# Barley yellow dwarf resistance in cereal crops: genetics and resistance mechanisms

by

**Shormin Choudhury**

B. Sc. Ag. (Hons.) and MS in Horticulture

Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

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The following people and institutions contributed to the publication of work undertaken as part of this thesis:

| Author | Name | Institution |
|---|---|---|
| Candidate | Shormin Choudhury | University of Tasmania |
| Author 1 | Meixue Zhou | University of Tasmania |
| Author 2 | Holger Meinke | University of Tasmania |
| Author 3 | Philip Larkin | CSIRO Agriculture and Food |
| Author 4 | Sergey Shabala | University of Tasmania |
| Author 5 | Craig Birchall | University of New England |
| Author 6 | Dhahi Turki Jadah Al-Shammari | University of New England |
| Author 7 | Guy Westmore | Biosecurity Tasmania |
| Author 8 | Rugen Xu | Yangzhou University |
| Author 9 | Matthew Hayden | La Trobe University |
| Author 10 | Kerrie Forrest | La Trobe University |
| Author 11 | Yun Fan | University of Tasmania |
| Author 12 | Peter Johnson | University of Tasmania |
| Author 13 | Hongliang Hu | University of Tasmania |
| Author 14 | Ibrahim Ahmed | University of Tasmania |
| Author 15 | Md Hasanuzzaman | University of Tasmania |

## Author Details and Their Roles

**Paper 1: Choudhury S,** Hu H, Meinke H, Shabala S, Westmore G, Larkin P and Zhou M (2017) Barley yellow dwarf viruses: infection mechanisms and breeding strategies. *Euphytica* **213**: 168---**Located in Chapter 2**

**Candidate was the primary author and** contributed to 50% of this work, including most of the literature collecting, rephrasing, and paper writings. Author 1 contributed to the literature collecting and writing. Author 2, Author 3, Author 4 and Author 5 contributed to the revision of the manuscript. Author 6 contributed to the proofreading and revision of the manuscript.

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**Candidate was the primary author and** contributed 80% to conduct the bulk experiments and wrote the paper draft. Author 1 and Author 2 contributed to experimental work. Author 3 and Author 4 contributed to the conception design the experiments and revision of the manuscript. Author 5 and Author 6 contributed to data interception and took the leading role in writing. Author 7 contributed to provide logistic support for this experiment, writing, proof-reading and revision of the manuscript.

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**Candidate was the primary author and** contributed 80% of the experimental work, conception, design the experiment and wrote the paper draft. Author 1 contributed to revision of the manuscript. Author 3, Author 4 and Author 5 contributed to provide logistic support for this experiment, data interpretation, writing, proof-reading and revision of the manuscript. Author 6 contributed to experimental work. Author 7 contributed to the design the experiment, critically assessed all data and commented proof-reading and revision of the manuscript.

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**Candidate was the primary author and** contributed 80% of the experimental work, conception, design the experiment and wrote the paper draft. Author 1 and Author 2 contributed to data interception and took the leading role in writing. Author 3 and Author 4 contributed to experimental work and revision of the manuscript. Author 6 contributed to provide logistic support for this experiment, writing, proof-reading and revision of the manuscript.

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**Candidate was the primary author and** contributed 80% of the experimental work and wrote the paper draft. Author 1, Author 3 contributed to data interception and took the leading role in writing. Author 2, Author 4 and Author 5 contributed to revision of the manuscript. Author 6 contributed to experimental work. Author 7 contributed to provide logistic support for this experiment, writing, proof-reading and revision of the manuscript. Author 8 contributed in data analysis and revision of the manuscript.

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**Candidate was the primary author and** contributed 80% of the experimental work and wrote the paper draft. Author 1 contributed to phenotypic work. Author 2 contributed to data interception and took the leading role in writing. Author 3 contributed to genotypic work. Author 4, Author 5, Author 6, Author 7 and Author 8 contributed to revision of the manuscript. Author 9 contributed to provide logistic support for this experiment, writing, proof-reading and revision of the manuscript.

We the undersigned agree with the above stated "proportion of work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

Signed: _____ _____

| | |
|---|---|
| *Prof. Dr. Meixue Zhou* | *Prof. Dr. Holger Meinke* |
| *Supervisor* | *Head of School* |
| *Tasmanian Institute of Agriculture* | *Tasmanian Institute of Agriculture* |
| *University of Tasmania* | *University of Tasmania* |
| Date: 3/12/2018 | Date: 5/12/2018 |

# Publications Arising from this Thesis

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*Shormin Choudhury*
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# List of Abbreviations

AAP, acquisition access period

ANOVA, analysis of variance

BaMMV, barley mild mosaic virus

BYD, barley yellow dwarf

BYDV, barley yellow dwarf virus

CC, companion cell

cM, centi-Morgans

CYDV, cereal yellow dwarf virus

DAS, double-antibody sandwich

DH, double haploid

DMF, dimethylformamide

dsRNA, double stranded RNA

ELISA, enzyme-linked immunosorbent assay

EST, express sequence tags

FW, fresh weight

GLM, general linear model

GWAS, genome-wide association study

hp, hairpin

IAP, inoculation access period

ICTV, International Committee on Taxonomy of Viruses

IM, interval mapping

IPP, infected phloem parenchyma

ISE, infected sieve elements

LISE, little infected sieve elements

LOD, logarithm of the odds

MAS, marker assisted selection

MLM, mixed linear model

MP, movement protein

MQM, multiple QTL model

MS, methyl salicylate

NE, Nuclear envelop

NSE, necrotic sieve elements

NSW, New South Wales

OD, optical density

PAL, phenylalanine ammonia lyase

PAR, photosynthethically active radiation

PCR, polymerase chain reaction

PLRV, potato leaf roll luteovirus

PRSV, papaya ring spot virus

PTGS, post-transcriptional gene silencing

Q-Q, quantile-quantile

q-RT-PCR, quantitative real-time reverse-transcription polymerase chain reaction

QTL, quantitative trait locus

RCBD, randomized complete block design

RdRp, RNA dependent RNA polymerase

RGAP, quresistance gene analog polymorphism

RGAs, Resistance gene analogs

RILs, recombinant inbred lines

RNAi, RNA interference

SA, salicylic acid

SBWMV, soil-borne wheat mosaic virus

ScYLV, sugarcane yellow leaf virus

SE, sieve elements

SgRNA, subgenomic RNA

SNP, single nucleotide polymorphism

SuCMoV, sunflower chlorotic mottle virus

TAS, triple-antibody sandwich

TBIA, tissue blot immunoassay

TKW, thousand-kernel weight

TMV, tobacco mosaic virus

TYLCV, tomato yellow leaf Curl Virus

VSRs, viral suppressors of RNA silencing

VSS, visual symptom score

WAI, weeks after infection

WDV, wheat dwarf virus

WSMV, wheat streak mosaic virus

WSSMV, wheat spindle streak mosaic virus

YVMV, yellow vein mosaic virus

# Abstract

Barley yellow dwarf (BYD) is one of the most widespread and serious viral diseases in the world. The causal agent, Barley yellow dwarf virus (BYDV) can infect cereal crops including wheat, barley and oats, leading to significant yield losses. There are several strains of BYDV, among the BYDV strains (PAV, MAV, SGV, RPV and RMV), BYDV-PAV is the most common and prevailing strain in Australia. Breeding BYD resistant/tolerant crops has become one of the top priorities for controlling BYDV. A proper resistance screening method is crucial for selecting resistant genotypes in a breeding program. In this study, we developed a reliable screening method for BYDV-PAV resistance of cereal crops under glasshouse conditions. At two-leaf stage, inoculation of 5-10 viruliferous aphids per plant for four days was shown to be a quick and effective screening technique for selecting BYD resistance in wheat, barley and oats. Visual evaluation of symptoms on barley and oats is considered adequate for evaluating BYD resistance. For wheat, it is necessary to assess BYD resistance by enzyme-linked immunosorbent assay (ELISA) or tissue blot immunoassay (TBIA) and measuring plant biomass (at early stage) and grain number and yield (at late stage).

To gain a better understanding of plant defence mechanisms of BYD resistance genes (*Bdv2* and *Ryd2*) against BYDV-PAV infection, we investigated the differences in agronomical, biochemical and histological changes between susceptible and resistant wheat and barley cultivars. Following BYDV infection, root growth and total dry matter of susceptible genotypes showed greater reduction than those of resistant genotypes. Virus multiplication in the phloem resulted in altered allocation of sugar, i.e. reduced sugar transport and accumulation of sugars, and altered leaf ultrastructure, coupled with necrosis in vascular bundles. Increased production of phenolic compounds may play a role in the resistance and defensive mechanisms of both *Bdv2* and *Ryd2* against virus infection.

We compared different physiological measures such as gas exchange parameters, quantum yield of PSII (chlorophyll fluorescence Fv/Fm ratio), chlorophyll content, dry biomass, leaf area and relative water, and yield attributes and pasting properties under BYDV stress. We used four wheat genotypes subjected to different BYDV treatments under field conditions to determine any differences for these traits

between susceptible and resistant genotypes under BYDV infection. We also confirmed BYDV infection using TBIA. Pasting properties were hardly affected by BYDV infection, with genotype having a larger effect than infection. Grain yield showed positive correlations with all gas exchange parameters, chlorophyll fluorescence, chlorophyll content, leaf area, relative water content and biomass weight; grain yield negatively correlated with TBIA and visual symptom scores. The results suggest that stomatal conductance, transpiration rate and chlorophyll fluorescence measurements are suitable for assessment of BYDV infection and screening BYD susceptible and resistant wheat genotypes.

Identifying new resistance genes/quantitative trait locus (QTL) is an essential, first step in the development of breeding-based control mechanisms of barely yellow dwarf virus. A genome-wide association study (GWAS) was performed on 335 wheat accessions using a recently developed, high-density wheat single nucleotide polymorphism (SNP) array. All accessions were assessed for BYD resistance under different environments. Marker-trait associations were performed using a general linear model (GLM) and a mixed linear model (MLM). A total of 36 significant marker-trait associations were identified, four of which arose consistently across three models. Five novel QTL on chromosomes 2A, 2B, 6A 7A and 7B, with the nearest markers of IWA3520, IWB24938, WB69770, IWB57703 and IWB65432, respectively were consistently detected in two models. This was the first GWAS study on BYD resistance in wheat accessions. Several wheat genotypes showed consistent resistance in different field trials. None of these genotypes contained *Bdv2*, *Bdv3* or *Bdv4* gene. These genotypes will be used in our further research to confirm the QTL identified in this research or map new QTL for BYD resistance.

A double haploid (DH) population from the cross between XuBYDV (introduced from China and showed very good resistance to BYD) and H-120 (also introduced from China but BYD sensitive) was used to identify new QTL for BYD resistance. This population was genotyped using a high-density wheat SNP array containing iSelect 90K SNPs. Each plant of the DH lines was inoculated with 5-10 viruliferous aphids for four days. Disease resistance of BYDV inoculated DH lines was assessed at heading stage and BYDV infection was tested by Tissue blot immunoassay (TBIA). Three new significant QTL were identified on chromosomes 5A, 6A and 7A for both symptom score and TBIA score, all three resistance alleles being from

XuBYDV. Some lines with resistance alleles from these three QTL showed high level resistance to BYD. These new QTL will be useful in breeding programs for pyramiding BYD resistance genes.

In conclusion, several QTL were identified for BYD resistance based on visual symptom score and TBIA score. The QTL identified for TBIA score and symptom score were located at different positions to those for BYD resistance. A total of five significant QTL were identified through genome wide association studies. Some of the genotypes in the study showed similar or even better resistance to BYD than those genotypes with known resistance gene (*Bdv2*). These genotypes and the five identified QTL will be useful for breeders to generate combinations with and without *Bdv2* to achieve higher levels more stable BYD resistance.

# Chapter 1. General Introduction

## 1.1 Background

### 1.1.1 Plant response to barley yellow dwarf disease and its tolerance mechanisms

Barley yellow dwarf (BYD), is one of the major viral diseases of small grains throughout the world and in Australia. It reduces yields by impacting on yield components such as heads number per plant, number of grains per head and weight of grain (McKirdy *et al.*, 2002). Yield losses can range from 5% to 80% (Perry *et al.*, 2000). In California, barley yellow dwarf (BYD) was first identified in barley crop in 1951 (Oswald and Houston, 1953) and later recognized in oat and wheat. There are several strains of BYDV, among them barley yellow dwarf virus- PAV (BYDV-PAV), which belongs to the genus Luteovirus of the family Luteoviridae; it is the most common serotype (Griesbach *et al.*, 1990). BYDV-PAV is transmitted by the aphids *Rhopalosiphum padi* and *Sitobion avenae* (Kaddachi *et al.*, 2014). BYDV causes physiological, biochemical and ultrastructural changes, including the disruption of photosynthate transportation, deterioration of phloem, and the development of specific ultrastructural inclusions (Gill and Chong, 1975). Symptoms of BYDV infection comprise leaf discoloration, such as reddening in oats, yellowing in barley and yellowing or reddening in wheat genotypes (Kosova *et al.*, 2008). Visual symptoms begin from the tip of the leaf and spread to the base of leaf. Plant growth and development are also affected by BYDV infection as it reduces plant biomass, vigour, and delays heading date.

Several approaches are available for controlling BYDV infection. The aphid vector can be effectively controlled through insecticide treatments, but the timing is critical, and several applications can make this method expensive and environmentally damaging (Chain *et al.*, 2005). Plant genotypes comprising genetic tolerance/resistances is one of the most effective, economical, sustainable and frequently employed strategies for controlling viral infections (Nicaise, 2014). Virus tolerance describes the ability of a plant genotype to endure or recover from the destructive effects of the virus infection. Tolerant plants have lower yield reduction and due to mechanism, that restrict or prevent virus infection in a host species (Cooper and Jones, 1983). For reliable selection of BYD tolerant/resistant

genotypes uniform inoculation with viruliferous aphids is essential. In the field, natural infections can be too unreliable for effective breeding progress. Controlled inoculation by infestation with viruliferous aphids also enables assessment of specific virus isolates and inoculation impacts at plant developmental stages. While feeding on plant tissue, viruliferous aphids transmit the virus with its saliva through the proboscis to the phloem (Ng and Perry, 2004). Initially, virus spread from cell-to-cell (short distance movement); in later stage, viruses are transported quickly in phloem cells, mentioned to as long-distance movement (Hipper *et al.*, 2013; Waigmann *et al.*, 2004).

Virus infection can alter the whole-plant metabolite profiles (Xu *et al.*, 2008; Shalitin and Wolf, 2000). Many plant defence pathways can be stimulated or suppressed by virus infection (Lewsey *et al.*, 2010; Whitham *et al.*, 2006). In plants, strong metabolic and ultrastructural alterations can occur by virus infection, even when no visual symptoms are apparent (Yan *et al.*, 2008). In host plants, disease progress is likely to induce significant biochemical alterations i.e. protein, phenolics, carbohydrates, and these metabolic alterations may favour or obstruct disease progress (Ayres *et al.*, 1996). In cell walls, the synthesis of phenolics and their polymerization has been suggested as plant defence response to infection (Sattler and Funnell-Harris, 2013), and low levels of phenolics may be related to disease susceptibility (Yao *et al.*, 1995). Sugar metabolism is a dynamic method with both metabolic fluxes and sugar concentrations altering dramatically during plant development and in response to environmental signals such as diurnal alterations and biotic stresses (Bläsing *et al.*, 2005; Borisjuk *et al.*, 2003). Due to virus infection, the amount of sugar significantly increased within the phloem (Shalitin and Wolf, 2000). Reduction of sugar translocation and other nutrient molecules to the root system restricts root growth and function, affecting plant growth and grain yield (Riedell *et al.*, 2003). In breeding, biochemical response helped with the selection of disease resistant genotypes for many crops (Lattanzio *et al.*, 2006).

It is important to identify the most suitable physiological parameter to evaluate for BYD resistance. Plant grain yield will be the ultimate test but requires that the entire life cycle is assessed in the field. This is time-consuming and labour-intensive. Physiological traits such as Photosynthesis, transpiration rate, stomatal conductance,

$CO_2$ assimilation, chlorophyll content, Fv/Fm and relative water content would be useful, physiological parameters to measure. The variation of physiological processes due to viral diseases is one of the most important causes of decreased crop productivity across the world (Agrios, 1997). Yield reductions following virus infection might be due to decreased photosynthesis. The mechanisms through which viruses induce the reduction of photosynthesis and other physiological traits in host plants are not fully understood, nor are the mechanisms of BYD resistance and tolerance. Photosynthesis might be impacted by reduction in chloroplast number and loss of chlorophyll content in various viral infections (Balachandran *et al*., 1997; Ryšlavá *et al.*, 2003; Guo *et al*., 2005a), with gas exchange parameters and chlorophyll fluorescence being possible, suitable indicators (Bonfig *et al.*, 2006; Berger *et al.*, 2007). Chlorophyll fluorescence and, mainly, the maximum quantum efficiency of light harvesting in PSII in dark adapted leaves, called Fv/Fm ratio, have proved to be a reliable indicator for abiotic and biotic stress tolerance (Duraes *et al.*, 2001). A significant decrease in Fv/Fm was observed in Nicotiana tabacum leaves infected by potato virus A and potato virus Y (Ryšlavá *et al.*, 2003; Zhou *et al.*, 2004). However, this is not always the case as Eupatorium makinoi plants infected by a geminivirus showed no significant changes in the Fv/Fm ratio (Funayama *et al*., 1997a). Despite the importance of BYD, little attention has been given to physiological traits as potential selection criteria for resistance breeding.

Cereal crops are vulnerable to BYDV infection when plants are infected at early growth stages. To introduce BYD resistance genotypes in cereal crops, it is important to develop a rapid and reliable detection methods that enable to identify the genotypes with resistance genes and to define the best selection criteria contributing to yield reduction owing to BYDV infection.

### 1.1.2 Genetic approach for breeding BYD tolerant plants

Leaf discolorations commonly found in plants infected by BYDV are often used for selecting BYD resistance plants, with the development of resistant wheat, barley and oat genotypes on the basis of this method (Kosova *et al.*, 2008; Veskrna *et al.*, 2009). Similar symptoms may also be caused by some other biotic or abiotic stresses. This complicates selection based on visual symptoms alone. However, tissue blot immunoassay (TBIA) and enzyme linked immunosorbent assay (ELISA)

have been used for identifying virus strains and their development, overcoming the limitation of visual assessment. Evaluation of wheat for BYD resistance using viruliferous aphid inoculation and ELISA analyses is very laborious and costly. Molecular marker assisted selection (MAS) would allow a quicker progress in breeding wheat for BYD resistant.

Direct selection for BYD resistance is affected by environmental factors and is therefore largely ineffective. Molecular markers would be a great help in achieving this combination because in most segregating population it can easily be scored and mapped (Kearsey and Farquhar, 1998). Molecular markers have been successfully used for tagging genes or quantitative trait locus (QTL) (Petersen *et al.*, 2015). Bi-parental mapping populations are normally used for determining the locations of resistance genes or QTL in cultivars (Letta *et al.*, 2013). BYD tolerance is polygenic in wheat (Cisar *et al.*, 1982; Tola and Kronstad, 1984), barley (Schaller, 1984; Qualset, 1992) and oats (McKenzie *et al.*, 1985). Genomic regions involved in tolerance to BYD were mapped in oats (Jin *et al.*, 1998; Barbosa-Neto *et al.*, 2000) and barley (Toojinda *et al.*, 2000). No effective BYD resistance gene has been found in bread wheat (Ayala *et al.*, 2001). *Bdv1* is the only gene for BYD tolerance in bread wheat (Singh *et al.*, 1993), located on 7DS (Ayala *et al.*, 2002). Three BYD resistance genes from *Thinopyrum intermedium* (intermediate wheatgrass) called *Bdv2*, *Bdv3* and *Bdv4*, have been introgressed into common wheat background via chromosomal translocations (Zhang *et al.*, 2009). *Bdv2* was first introduced as a 7D-7Ai#1 translocation (Banks *et al.*, 1995). *Bdv3* is on a 7B-7Ai#1 translocation (Crasta *et al.*, 2000). *Bdv4* was first identified on a group 2 chromosome from Th. intermedium (Larkin et al., 1995) and subsequently developed as a 2D-2Ai-2 translocation (Zhang *et al.*, 2009). There are evidently different BYD resistance genes in Th. intermedium with different isolate specificities (Francki *et al.*, 2001) and possibly diverse mechanisms of action, such as interfering with virus multiplication (Sharma *et al.*, 1989) or reducing cell-to-cell movement (Anderson *et al.*, 1998).

*Bdv2* was the first BYD resistance gene for wheat breeding. Three BYD-resistant wheat varieties were developed from *T. intermedium* and released in Australia and China, respectively (Zhang *et al.*, 2009). In Australia, two cultivars, namely Mackellar (derived from TC14) and Glover (derived from TC6), have been released,

both carrying the resistance gene *Bdv2* (Ayala-Navarrete *et al.*, 2007). The availability of different molecular tools allows for the characterization of genes of interest and identification of plants carrying the target genes. This will improve conventional breeding efficiency. To identify BYD resistance genes and linked QTL, molecular dissection will now be faster. Genomic selection will also allow genotypes to be developed with major and minor effects with effective combinations of multiple loci, hopefully attaining better resistance and more durable resistance. Such marker assistant breeding avoids all the difficulties associated with rearing the viruliferous aphids and having disease progress confounded by environmental factors. While effective sources of resistance to BYD have been identified in wheatgrasses, few have been reported in common wheat (Ayala-Navarrete and Larkin, 2011). Therefore, to find new resistance sources for wheat breeding programs, the search needs to continue to strengthen and broaden the defences against this prevalent and destructive BYD disease.

## 1.2 Objectives and research aim

The following specific objectives were addressed:

**(1) Evaluate suitable screening technique for BYD resistance in cereal crops**

For effective breeding progress, natural infections in the field can be too unreliable. To select potentially promising BYD resistant genotypes in cereals breeding programme, the availability of efficient screening tools is important. A technique that can give a rapid and reliable detection of the resistance genes in the germplasm under BYDV stress is required. Therefore, the objectives of this research were to quantify plant growth and yield characteristics of wheat, barley and oat varieties under BYDV infection, and to assess the suitability of different screening approaches.

**(2) Understand the agronomical, biochemical and histological response of resistant and susceptible cereal crops under BYDV stress**

In barley and wheat, many studies were conducted with BYD resistance but little information has been reported about the alterations of biochemical compounds under BYDV infection. Thus, it is important to evaluate the response of susceptible and resistant wheat and barley plants under various agronomical, biochemical and

cell ultrastructural changes after systemic BYDV infection. This enables detection of desirable, key traits of BYD resistance mechanism via glass house experiments.

**(3) *Barley yellow dwarf virus* infection affects physiology, morphology, grain yield and flour pasting properties of wheat**

Different crops have developed multiple mechanism to cope with biotic stresses. However, there are very few studies on mechanisms encoding BYD resistance in wheat. Thus, it is important to understand physiology and morphology mechanism encoding BYD resistance under plant growth, yield and pasting properties level.

**(4) Conduct genome wide association studies of resistance to BYD disease in wheat**

Very few resistance genes/QTL have been reported for BYD resistance in wheat. To find BYD resistance genes/QTL from wheat collections, we have collected over 300 wheat accessions worldwide. These accessions showed a wide variation in BYD resistance, from resistant to very susceptible. A genome wide association study (GWAS) was conducted on a geographically diverse collection of 335 bread wheat accessions to identify new sources of resistance to BYD.

**(5) Identification of new QTL contributing to barley yellow dwarf virus resistance in wheat**

A double haploid (DH) population from the cross between XuBYDV (introduced from China, showing very good resistance to BYD) and H-120 (also introduced from China but BYD sensitive) was used. In the present study, we used SNP markers and DH population to identify QTL controlling resistance to BYDV stress. TBIA score and symptom severity under BYDV stress were used as the indicators of BYD resistance.

## 1.3 Outline of the chapters

Chapter 2: Literature review about Barley Yellow Dwarf Viruses: Infection Mechanisms and Breeding Strategies

Chapter 3: A screening method to detect BYDV-PAV resistance in cereals under glasshouse conditions

Chapter 4: Agronomical, biochemical and histological response of resistant and susceptible wheat and barley under BYDV stress

Chapter 5: Barley yellow dwarf virus infection affects physiology, morphology, grain yield and flour pasting properties of wheat

Chapter 6: Genome wide association study reveals novel genes for barley yellow dwarf virus resistance in wheat

Chapter 7: Identification of new QTL contributing to barley yellow dwarf virus resistance in wheat

Chapter 8: General discussion and conclusions

# Chapter 2. Literature Review

## Barley Yellow Dwarf Viruses: Infection Mechanisms and Breeding Strategies[1]


### Abstract

Barley yellow dwarf virus (BYDV) infection often results in substantial yield losses in susceptible cereal crops. Major symptoms of BYDV infection in cereals include plant dwarfing and colour changes of leaf blades along the vascular bundles, especially of leaf tips. A full understanding of physiological and molecular mechanisms contributing to resistance provides salient information for breeding BYD resistant varieties and developing strategies to address the problem. In this paper, we reviewed BYDV infection mechanisms and summarised current information on known resistance genes, molecular markers and the use of transgenic techniques in breeding of BYD resistant varieties. Cereal yellow dwarf viruses (CYDVs) are also discussed as both BYDV and CYDV belong to the family *Luteoviridae*.


### 2.1 Introduction

Barley yellow dwarf is one of the most widespread and serious viral diseases in the world (Ayala *et al.*, 2001; Ayala-Navarrete and Larkin, 2011). It causes a significant reduction in cereal grain production. In wheat, yield losses are estimated to be 13-25 kg/ha (McKirdy *et al.,* 2002) or even 27-45 kg/ha (Banks *et al.*, 1995) for each 1% increase in the incidence of the virus species. Up to 80% yield loss of cereal crops due to barley yellow dwarf virus (BYDV) or cereal yellow dwarf virus (CYDV) infection has been reported, which is caused by reduced numbers of tillers per plant, seeds per tiller, and seed weight (McKirdy *et al.,* 2002; Perry *et al.*, 2000).

Barley yellow dwarf (BYD) was first recognized in California in 1951 in barley (Oswald and Houston, 1953) and later identified in oat and wheat. The disease is

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caused by any one or a complex of closely related viruses comprising two main genera: barley yellow dwarf *Luteovirus* (BYDV) and cereal yellow dwarf *Polerovirus* (CYDV) in the family *Luteoviridae* (Liu *et al.*, 2007). Hereafter we will refer to the complex as BYDV, unless needing to be more specific. BYDV is transmitted by a number of aphid species (D'arcy, 1995), is propagated progressively (Chain *et al.*, 2005) and causes physiological, biochemical and cytological alterations, including the restriction of photosynthate transportation, phloem degeneration, the creation of a nutritionally enhanced phloem diet for the aphid vectors (Telang *et al.*, 1999), and the formation of specific cytological inclusions (Gill and Chong, 1975). Symptoms for BYDV infection on cereals include leaf discoloration, stunting of the plants and reduced number of tillers, kernels per spike, kernel weight and root growth (Riedell *et al.*, 2003). This results in grain yield reductions and, in severe cases, the infection can lead to the death of plants.

BYD can be controlled partially through management practices such as sowing date and the application of insecticides but breeding for resistant cultivars is the most effective and environmentally sound approach to prevent yield losses. BYD resistant genotypes can be identified by low virus concentrations (Cooper and Jones, 1983) estimated via the enzyme-linked immunosorbent assay (ELISA), tissue blotting immunoassay (TBIA) and quantitative real-time reverse-transcription polymerase chain reaction (q-RT-PCR). Significant efforts have been made to identify molecular markers linked to BYD resistance genes. PCR markers are the most suitable markers for detecting the presence of resistance genes in a very small amount of plant material. The identification of genome regions for BYD resistance and further application of this knowledge in marker assisted selection (MAS) would enable faster progress in cereal crop breeding.

## 2.2 Symptoms of BYD

BYDV infection can lead to phloem degradation and collapse of sieve elements. Reduction in plant growth (dwarf appearance) and colour changes of leaf blades along the vascular bundles, especially of leaf tips (Kosova *et al.*, 2008) are the main symptoms. Barley leaves often turn bright yellow while oat leaves may become

orange or red, and wheat leaves typically turn yellow or red after infection. BYDV interfere with physiological processes resulting in chlorosis and stunting (Jensen and D'Arcy, 1995). Other symptoms may include serrations along leaf borders and leaf folding into corkscrew patterns. Four to twelve days after inoculation the symptoms of virus invasion start to appear (Makkouk *et al.*, 1994). Furthermore, several economically related traits are reduced including the number of florets per head (Hoffman and Kolb, 1998; Jensen *et al.*, 1971), the number of ears per plant and kernel weight (Jarošová *et al.*, 2013). Virus infection also delays heading date (D'Arcy, 1995).

Roots play an important role in plant growth and development, providing water and nutrients from the soil to the growing plant. High virus accumulation in roots has been observed 9–15 days after inoculation (Šíp *et al.*, 2006). BYDV infection severely reduces root length, distance from seminal root tip to the nearest lateral root, and the root to shoot ratio, due to lower water and nutrient uptake (Riedell *et al.*, 2003). More than 40% reduction in root length has been reported in spring wheat infected by *Rhopalosiphum padi* transmitted BYDV (Kolb *et al.*, 1991; Riedell *et al.*, 2003). However, while both root and shoot dry matter was reduced with BYDV infection, there was an increase in the induction of adventitious roots for some cultivars (Hoffman and Kolb, 1997). The root system in the early stage of infection is affected more severely than the shoot in BYDV-infected wheat seedlings (Hoffman and Kolb, 1997), with root showing stunting at 4 days after inoculation, when no chlorosis and stunting were seen in shoots. Stunting of the root system usually precedes visual symptoms in the shoot by 7-10 days in barley, wheat, and oat. This is mainly attributed to a reduction in the length of the root system rather than a reduction in the number of roots.

## 2.3 Viral genome organisation and function

Barley yellow dwarf viruses are 25 nm icosahedral (T=3) particles consisting of a major ~22 kDa coat protein and a minor ~52 kDa component encapsidating a 5.7 kb, single-stranded positive sense RNA (D'Arcy, 2000), which contains a 5'-linked protein (VPg) and no poly (A) tail. The nucleotide sequence of the genomic RNA of BYDV was determined (Miller *et al.*, 1988b). In the genome there are six open

reading frames (ORFs). Only ORFs 1 and 2 are translated from genomic RNA whereas ORFs 3, 4, and 5 all are translated from subgenomic RNA (sgRNA1). Among these, the 5′-proximal ORFs are the only genes necessary for BYDV RNA replication in protoplasts. ORF2 encodes the RNA-dependent RNA polymerase (RdRp). It is only expressed via minus one (−1) ribosomal frameshifting in the region of overlap fused to ORF1 (Paul *et al.*, 2001). For BYDV-PAV, ORF1 and ORF2 are probably required for virus replication in oat as no genomic or subgenomic RNAs were detected in cells inoculated with deletion or insertion mutants in these regions (Mohan *et al.*, 1995). ORF3 encodes a major coat protein (CP) of 22 kDa (Miller *et al.*, 1988a). The coat protein plays a key role in maintaining a high level of accumulation of genomic RNA, though unnecessary for PAV replication (Mohan *et al.*, 1995). ORF4 is entirely nested within ORF3 and codes for a 17 kDa non-structural protein required for BYDV-PAV to spread systemically in plants (Chay *et al.*, 1996). The expression of ORF 4 is associated with a unique regulatory mechanism of ribosome leaky scanning mechanism (Dinesh-Kumar and Miller, 1993). The ORF4 translation product is similar to that of the homologue of ORF4 in potato leafroll *Polerovirus* (PLRV), which has biochemical properties specific to known movement proteins, including the ability to be phosphorylated, binding non-specifically to nucleic acids (Tacke *et al.*, 1991; 1993) and localisation to the plasmodesmata (Schmitz *et al.*, 1997). PAV ORF5 is fused to CP as a read-through domain and encodes a 50 kDa protein expressed as a 72 kDa fusion protein via a read-through suppression of the ORF3 stop codon (Cheng *et al.*, 1994; Dinesh-Kumar *et al.*, 1992; Filichkin *et al.*, 1994; Wang *et al.*, 1995). A frameshift mutation within ORF6 was reported to be incompatible with BYDV-PAV RNA replication in protoplasts (Young *et al.*, 1991). Mohan et al (1995) found that the RNA sequence encoding or flanking ORF6, rather than the protein product of ORF6 is required for PAV replication in oat protoplasts.

## 2.4 Taxonomy and transmission of BYDV

The International Committee on Taxonomy of Viruses (ICTV) (2016) classifies BYDV and CYDV into two independent genera: Luteovirus and Polerovirus, respectively, both in the family Luteoviridae. BYDV comprises three species, Barley yellow dwarf virus-PAV (BYDV-PAV), Barley yellow dwarf virus-MAV

(BYDV-MAV) and Barley yellow dwarf virus-PAS (BYDV-PAS), based on differences in vector specificity and serology. CYDV comprises two species, Cereal yellow dwarf virus-RPV (CYDV-RPV) and Cereal yellow dwarf virus-RPS (CYDV-RPS) (Rochow, 1969; Rochow and Muller, 1971). BYDV-SGV, -GPV and -RMV have also been identified not yet been assigned to either genera (Van Regenmortel *et al.*, 2000; Wu *et al.*, 2011). With currently accepted sequence-based species demarcation criterion which requires more than 10% amino acid sequence divergence in any of the viral gene products (Wu *et al.*, 2011), Chinese isolates of BYDVs were divided into four species following Rochow's system, namely BYDV-GAV, -GPV, -PAV, and -RMV (Liu *et al.*, 2007; Zhou *et al.*, 1987). Virus isolates identified as BYDV-PAV were recently separated into three distinct subspecies, BYDV-PAV (PAV-I), -PAS (PAV-II) and PAV-CN (PAV-III), based in part on antibody reaction, genomic sequences, and/or symptoms in various host plants. The three subspecies have more than 10% differences at the amino acid level for any viral gene product (Liu *et al.*, 2007). BYDV-PAV is the most prevalent and damaging species (Lister and Ranieri, 1995). Even though CYDV-RPV has worldwide distribution, it is less frequent and has relatively lower impact than BYDV-PAV does in small grains (Lapierre and Signoret, 2004).

BYDVs are not mechanically or seed transmissible, but are transmitted by aphids in a persistent, circulative and non-propagative manner (Miller *et al.*, 2002a). More than 25 aphid species are involved in the transmission of viruses (Halbert and Voegtlin, 1995). *Rhopalosiphum padi* (bird-cherry-oat aphid), *Sitobion avenae* (English grain aphid), *Rhopalosiphum maidis* (corn leaf aphid), *Schizaphis graminum* (green bug) are major vectors of BYDVs (Chapin *et al.*, 2001). Each virus species is transmitted preferentially by specific aphid species. BYDV-MAV, CYDV-RPV (formerly BYDV-RPV), BYDV-RMV and BYDV-SGV are transmitted most efficiently by *Sitobion avenae* (formerly *Macrosiphum avenae*), *R. padi*, *R. maidis* and *Schizaphis graminum*, respectively, while BYDV-PAV is transmitted efficiently by both *R. padi* and *S. avenae*. More recently, two new viruses, BYDV-GAV [transmitted by *S. graminum* and *S. avenae* (Huson and Bryant, 2006)] and BYDV-GPV [transmitted by *S. graminum* and *R. padi* (Wernersson and Pedersen, 2003)] have been reported. *R. padi* is an efficient vector

for both BYDV and CYDV, and is therefore the most damaging aphid species (Gourmet *et al.*, 1994).

The transmission of BYDVs is influenced by the duration of access periods and the availability of virus in source plants (Gray *et al.*, 1991). Maximum transmission efficiency of BYDV is achieved with an acquisition access period (AAPs) of 48 h (Jimenez-Martinez and Bosque-Perez, 2004) and inoculation access period (IAP) of 72 h (Parizoto *et al.*, 2013). Virus transmission occurs when aphids feed on the phloem sieve tube elements of host plants. Phloem cells become infected with BYDV when an aphid deposits the virus in its saliva with its stylet while feeding on sap. BYDV transmission is positively correlated with virus titre maintained in the aphids (Guo *et al.*, 1997).

Variation in BYDV transmission also depends on environmental factors. Temperature is the most significant environmental factor affecting virus transmission efficiency (Guo *et al.*, 1996; Stoutjesdijk *et al.*, 2001). For most aphid species, there is a strong, positive, linear relationship between temperature and development. Virus transmission rates are also sensitive to variation in temperature. A temperature of 12 °C is usually recognized as the lowest temperature for aphid feeding or virus transmission (Ag PJDBB, 1991; Lowles *et al.*, 1996). Both PAV and RPV approximately double their transmission rates with an increase in temperature from 15 to 20 °C (Gildow, 1990). Different species also respond differently to temperature. CYDV-RPV-like isolates require 50 h for virus transmission, whereas BYDV-PAV-like isolates require 62 h at 15 °C. Both species require only 35 h for virus transmission at 20 °C (Parry *et al.*, 2012). Virus content in plants as well as virus stability within the feeding pattern of aphids and vector aphids are also potentially influenced by temperature (Lowles *et al.*, 1996).

## *2.5* Replication and propagation in host

Viruliferous aphids transmit the virus to plant phloem when saliva is injected through the proboscis during feeding (Ng and Perry, 2004). Viral infection usually starts with virus replication within an initially infected cell, followed by virus spreading to neighbouring cells through plasmodesmata, an intercellular conduit connecting through cell walls. The process is referred to as cell-to-cell (short-

distance) movement, facilitated by a special protein called viral movement protein (MP). In the latter phase termed systemic (long-distance) movement, plant viruses enter into the vascular tissue, distribute and unload into non-infected tissues, aided by the phloem stream (Waigmann *et al.*, 2004). It is generally presumed that the cell-to-cell movement is an active function, requiring specific interaction between the virus and plasmodesmata, whereas systemic viral spread through the vascular tissue is a passive process, driven by the flow of photoassimilates (Ghoshroy *et al.*, 1997). The discovery that a 30 kDa movement protein (MP) encoded by Tobacco mosaic virus (TMV) was required for viral cell-to-cell movement (Deom *et al.*, 1987; Meshi *et al.*, 1987) opened a new avenue to explore the trafficking mechanisms of a wider array of viruses. For some viruses, the role of viral MP in protein and RNA trafficking into the phloem and inter-organ regulation of plant development were thoroughly studied (Balachandran *et al.*, 1997; Haywood *et al.*, 2002). The MP can associate with the viral nucleic acid molecule and transport it through plasmodesmata. The 30 kDa protein (P30) of Tobacco Mosaic Virus (TMV) was the first viral MP discovered to be able to bind single-stranded nucleic acid specifically (Citovsky *et al.*, 1990), mediated by two independently active domains of the MP (Citovsky *et al.*, 1992). The P30-TMV RNA complex measures a diameter of 1.5 to 3.5 nm (Citovsky *et al.*, 1992; Kiselyova *et al.*, 2001) and may interact with the cytoskeletal elements to facilitate transport of the P30-TMV RNA complex from cytoplasm to plasmodesmata (Heinlein *et al.*, 1995; McLean *et al.*, 1995). The diameter is even smaller than a protein-free, folded TMV RNA, which allows easy access through dilated plasmodesmata (Waigmann *et al.*, 1994; Wolf *et al.*, 1989). The nucleic acid binding activity is also present in PLRV, which is a member of the genus Luteovirus and expresses a 17 kDa MP. The MP could bind non-specifically to single-stranded RNA and DNA in vitro (Tacke *et al.*, 1991) and associate with plasmodesmata in host plants (Schmitz *et al.*, 1997). This provides us a clue that ORF 4 proteins in luteoviruses may also assist virus cell-to-cell spread in host plants (Xia *et al.*, 2008) as there is a high similarity of amino acid sequence between ORF 4 protein encoded by luteoviruses and PLRV MP (Jin *et al.*, 2004; Miller *et al.*, 2002b). BYDV-PAV MP may also assist the transport of the viral genome into the nucleus as the MP is present in the cytoplasm and the nucleus (Nass *et al.*, 1998).

After entry into the cytoplasm, protein synthesis is initiated (Den Boon *et al.*, 2010). To protect themselves from host defences and enhance replication and transcription efficiency, viruses use the strategy of compartmentalization in specific intracellular components (Novoa *et al.,* 2005). Indeed, plant-infecting viral RNA genomes are known to be the most efficiently translated mRNAs (Fan *et al.,* 2012). A detailed description of BYDV translation mechanism is discussed by Ali *et al.* (2014). The translation of the BYDV genome is initiated by a unique mechanism which has not yet been observed in other known RNA yet. Although the genome lacks a 5'cap and a poly (A) tail, the translation element (3' TE) between ORF5 and 6 can initiate efficient cap independent translation at the AUG proximal to the 5' end of the RNA (Wang *et al.*, 1997).The communication between the 3' TE and the 5' end to ensure ribosome entry on the mRNA was achieved by direct base-pairing of a stem loop in the 3' UTR with a stem loop in the 5' UTR to form a closed loop, which allows a sequence in the 3' UTR to confer translation initiation at the 5'-proximal AUG (Guo *et al.*, 2001). The replication of PAV RNA may be coupled to translation as defective RNAs harbouring various deletions were not replicated in trans by the co-inoculated wild-type helper genome (Mohan *et al.,* 1995).

Although BYDV is a systemic virus and can move to the root system within 20 h after inoculation (Eweida *et al.*, 1988), its replication is almost totally restricted within the plant phloem tissue (Irwin and Thresh, 1990; Jensen SG, 1995), i.e. phloem parenchyma cells, companion cells and sieve tubes. The restricted site of infection in phloem tissue is an important feature of the Luteoviridae, which includes Luteovirus, Polerovirus and Enamovirus (Van Bel, 2003). The systemic spread is suspected to be associated with vascular transport of virions due to the discovery of BYDV particles in vasculature samples (Gill and Chong, 1975; Jensen, 1969). The association between the long-distance movement of some viruses and viral gene expression emphasizes the key role of MP. For example, two proteins encoded by geminiviruses are responsible for long-distance movement and for movement of single-stranded viral DNA in and out of the nuclei (Noueiry *et al.*, 1994; Pascal *et al.*, 1994). However, studies of the function of putative luteoviral MPs remains limited (Xia *et al.*, 2008). A 17 kDa protein encoded by ORF 4 is required for BYDV-PAV to spread systemically in plants (Chay *et al.*, 1996; Miller

*et al.*, 1997a). The replication of plant virus genome occurs in the nucleus of host cells (Hanley-Bowdoin *et al.*, 1999). The genome of viruses must be transported into the nucleus by mechanisms requiring viral MP (Liu *et al.*, 2005). The intracellular transport of the viral genome may require association with viral MPs, as the MPs of BYDV-GAV and BYDV-GPV could bind viral RNA in vitro and were observed to associate with the nuclear envelop (NE) when expressed as a GFP fusion protein in insect cells (Liu *et al.*, 2005). The interaction between MP and NE is enhanced by one of the four α-helices in the N-terminal portion of BYDV-GAV MP, possibly via its capacity to penetrate the NE structure, though not essential for targeting MP to nuclear envelop (Liu *et al.*, 2005). Further study discovered that the BYDV-GAV MP possesses a novel nuclear envelop (NE) targeting domain at its N-terminal and an arginine-rich RNA binding element at its C-terminal end. The C-terminal RNA binding element is also present in the MPs of other BYDV strains and PLRV as demonstrated by combined deletion mapping and site-directed mutagenesis experiments (Xia *et al.*, 2008).

## 2.6 Anatomical and cytological alterations

The first electron microscopy study of the internal symptoms of BYDV infection was performed in 1957, when Esau (1957a; b) discovered phloem degeneration and callose deposition inside the phloem as the first visible internal symptom. The deposition of callose was observed to plug plasmodesmata (Comeau and Haber, 2002), which may impede the translocation of photosynthates. The abundance of callose deposition and the way it plugs plasmodesmata might be called the primary symptom, the very first cause of all further damage. The primary internal symptom of the disease occurs in the vascular tissues, specifically in the phloem. Secondary effects develop in various other tissues. The infection leads to a premature necrosis of the phloem which later spreads to the xylem and ground parenchyma and causes death of the apical meristem and external symptoms such as stunting and leaf chlorosis (Esau, 1957b). The phloem-limited virus not only causes damage to the phloem and associated plasmodesmata (Comeau and Haber, 2002), but also produces viral proteins which influence plasmodesmatal structure to allow the passage of viral genomes or virus particles. For example, the TMV MP accumulates

in plasmodesmata and alters the size exclusion limit of the channel (Wolf *et al.*, 1989).

Gill and Chong (1975) used electron microscope to observe the development of infection in oat leaves inoculated with an isolate of BYDV (6407). At 3 days, virus particles were initially found in a form of small clusters (virus-like particles, slender filaments, small vesicles and an amorphous material) in the cytoplasm, whereas in non-inoculated tissues, none of these was found. Three stages of infection were proposed by Gill and Chong (1975; 1976; 1979). At the first stage, densely staining material appeared in plasmodesmata and an amorphous substance and viral RNA containing filaments appeared in the host cytoplasm. At the second stage, filaments became visible in the nuclear pores. During this stage, the nuclear outline became distorted and massive clumping of heterochromatin occurs. At the last stage, viral particles were seen in the nucleus after disintegration of nuclear membrane. Only cells of phloem parenchyma, sieve elements and companion cells were infected with the virus, which could not be seen in the xylem or mestome sheath. The virus-like particles were characterised by their circular outlines, high electron opacity and their diameter. These observed events were later confirmed by another study using immunogold labelling to detect the expression of viral coat protein of BYDV-PAV (Nass *et al.*, 1995), except that viral particles were observed within areas containing filamentous material, possibly because different virus isolates were used. Similarly, electron microscopy of maize infected with the 27/77 isolate of BYDV revealed virus-like particles in the nuclei of companion cells, plasmodesmata connecting companion cells with mature sieve tubes, the lumen of mature sieve tubes and xylem tracheal elements (Eweida *et al.*, 1983).

Visual symptoms may lag behind microscopic changes. Microscopic observation identified most target cells to be infected six days after inoculation, whereas symptoms on leaves appeared 12-14 days after inoculation (Chain *et al.*, 2005). Plants infected by sugarcane yellow leaf virus (SCYLV), even when visually non-symptomatic, underwent strong metabolic and ultrastructural changes (Yan *et al.*, 2008). Moreover, even in the absence of visual symptoms, significant economic damage may occur.

## 2.7 Physiological alterations

BYDV infection interferes with plant physiological processes, which cause dwarfing, chlorosis, stunting, and yield loss (Hoffman and Kolb, 1998; Jensen *et al.* 1971). BYDV is phloem tissue specific, where it replicates and moves mainly within phloem tissues (Barker and Harrison, 1986, Derrick and Barker, 1997). Virus infection was shown in all the companion cells, sieve elements, and the phloem parenchyma. Viruliferous aphids transmit the virus to plant phloem when saliva is injected through the proboscis during feeding (Ng and Perry, 2004). Phloem damage caused by BYDV limits the transport of photosynthate and restricts long-distance carbohydrate translocation. Carbohydrate accumulation in leaves consecutively inhibits photosynthesis, reduces chlorophyll and increases respiration (Jensen, 1968). The effects of BYDV infection on roots are more rapid and severe than on the shoots (Kolb *et al.*, 1991). Although root tips are far from the photosynthate source but suffered from reduced translocation. It is not well-known how root system function is affected by changes in root length or biomass caused BYDV infection. Because root systems provide shoot organs with essential mineral nutrients, potential reductions in root system function of BYDV-infected plants may play an important role in grain yield reductions (Riedell *et al.*, 2003). Due to BYDV infection a susceptible wheat cultivar showed 72% reduction in photosynthetic capacity. On the other hand, a moderately tolerant wheat cultivar exhibited only 60% reduction in photosynthesis (Jensen and Van Sambeek, 1972). Photosynthesis was reduced by 25% in per gram of fresh weight of tissue in BYDV-infected plants (Jensen *et al.*, 1971).

Chlorophyll is the most important pigment in plants and its concentration controls photosynthetic potential and primary production. BYDV infection reduced the average chlorophyll content by 80% in wheat (Jensen, 1972). Changes in total chlorophyll content of foliar tissue are important indicators of disturbed chloroplast development (Yoo *et al.*, 2009) that impaired photosynthetic capacity in plants exposed to a broad spectrum of biotic and abiotic stressors. Many studies have shown that virus infection can trigger severe chlorophyll breakdown within the host. An imbalance between biosynthesis and catabolic turnover of green pigments in

plant tissues indicates profound inhibition of photosynthesis process (Botha *et al.*, 2006; Diaz-Montano *et al.*, 2007; Goławska *et al.*, 2010; Heng-Moss *et al.*, 2003).

## 2.8 Biochemical alterations

The biotic stress triggered by virus infection leads to many biochemical changes. In the case of *Tobacco Mosaic Virus*, it was evidenced that the induction of phenylpropanoid compounds in the plant inhibits virus spread rather than virus multiplication (Massala *et al.*, 1980). Addition of inhibitors of phenylalanine ammonia lyase (PAL) increased the size of the lesions without changing viral titre. BYDV infected oats was shown to have significantly less fructans in the crowns, which has negative implications for survival through the winter (Livingston and Gildow, 1991). At the same time sucrose, glucose and fructose levels increased.

Virus infection also changes protein profiles in resistant wheat plants. BYDV susceptible wheat cultivars experienced significant reductions in protein content whereas resistant wheat maintained higher protein content throughout the duration of the infestation (Xu *et al.*, 2016). Synthesis or increased expression of specific plant proteins may serve to enhance the plant resistance to stresses (Ni *et al.*, 2001).

Jimenez-Martinez *et al.* (2004) demonstrated that BYDV triggers a change in the volatile organic compounds released in the headspace above plants, and that this headspace attracts more nonviruliferous aphids. BYDV-infection increased the concentration of total free amino acids in wheat leaves, notably in the phloem sap (Ajayi, 1986). This together with the increase in simple sugars may increase aphid feeding.

Phytohormone levels are also altered following BYDV infection. Davis *et al.* (2015) undertook a detailed study of abscisic acid, jasmonic acid, methyl jasmonate, methyl salicylate (MS), and salicylic acid (SA) following BYDV infection. They followed the phytohormones over time and with different watering conditions and compared to both undamaged controls and seedlings infested with non-viruliferous aphids. Total hormone concentrations in BYDV-infected plants were greater than those in sham-treated and control plants. SA was higher in infected plants but MS was lower.

Simple sugars (e.g. glucose, fructose, and sucrose) not only serve for carbon and energy sources, but also as signaling molecules (Bolouri-Moghaddam *et al.*, 2010; Rolland *et al.*, 2006). Soluble sugars in resistance responses to pathogens can stimulate isoflavone accumulation in plants as part of a defense mechanism (Morkunas *et al.*, 2005). In plant tissues, the levels of sugars increased due to virus infection including BYDV (Livingston and Gildow, 1991; Shalitin and Wolf, 2000). Therefore, plants can modulate their sugar pools for use as signals to increase immune reactions or for acting as a source of carbon and energy (Gómez-Ariza *et al.*, 2007). Compared with healthy plants, BYDV infected wheat plants altered amino acid composition. BYDV infection increased the total amino acid content of the sampled wheat leaves at different plant developmental stages with more increases at later stages (Ajayi, 1986). The total free amino acids were increased by from 150 to 180% in *Tomato spotted wilt tospovirus* infected tomato leaves compared with non-infected plants (Selman *et al.*, 1961).

Phenolic compounds are well known antifungal, antibacterial and antiviral compounds occurring in plants (Mandal *et al.*, 2010), often found in plants in the form of glycosides, which can be enzymatically converted to defensive substances at the site of attack (Kofalvi and Nassuth, 1995). Phenolic compounds may contribute to enhance the mechanical strength of host cell walls by the synthesis of lignin and suberin that are involved in the formation of physical barriers and can block the spread of pathogens (Ngadze *et al.*, 2012). Altering the level of phenolic compounds in plants changes disease susceptibility (Yao *et al.*, 1995). The inoculation of *cotton leaf curl Burewala virus* caused a decrease in the amount of total phenolic in susceptible genotypes but an increase in resistant genotypes (Siddique *et al.*, 2014). Similarly, Wheat Streak Mosaic Virus infection caused faster formation and accumulation of phenolic compounds in virus resistant wheat genotypes than those in susceptible ones (Sahhafi *et al.*, 2011).

## 2.9 Host-virus interaction: molecular basis

The infection and replication of virus in the host involves diverse mechanisms of interaction between host and virus. There are two basic types of host-virus interactions: compatible interactions that lead to host infection; and non-compatible

interactions that do not lead to host infection in the case of non-host or immune plants (Jarošová *et al.*, 2016). A compatible interaction can be either susceptible or resistant, distinct in the degree of virus multiplication within the host and severity of symptoms. Host resistance to many plant viruses is known to be sustained by elaborate gene networks that respond to biochemical triggers induced by virus inoculation (Cooper *et al.*, 2003; Genoud and Métraux, 1999). Molecular interruption of this network may be responsible for virus induced symptoms. In tomato, the hexose transporter *LeHT1* was found to be expressed preferentially in resistant plants and was demonstrated to be part of the network providing resistance *Tomato yellow leaf Curl Virus* (TYLCV) (Eybishtz *et al.*, 2010). The *LeHT1* silenced resistant plants showed enhanced expression of JNK-like proteins, which are components of the signalling cascade leading to stem necrosis (Zhang and Klessig, 2001), and had more active virus spread in the tissues and oxidative burst as part of an apoptotic mechanism. Studies on the link between BYDV activity and observed symptoms remain limited. However, Xia *et al.* (2012) demonstrated binding activity of the 17 kD movement protein of BYDV to form multimers with itself and proposed that this activity may be necessary for the induction of symptoms in plants. This follows an earlier study in which PLRV MP self-interacts to form dimers and induces yellowing and rolled leaf symptoms as evidenced by inoculation assays of PLRV mutants with defective MPs in potato (Lee *et al.*, 2002). The pathogenic function of BYDV MP dimerization requires further elucidation.

There appears to have been a long evolutionary arms race of developing defence and counter-defence strategies of plants and viruses against each other. One of the first innate immune responses which plant viruses encounter when invading a host is antiviral RNA silencing or RNA interference (RNAi) which is a form of post-transcriptional gene silencing (PTGS). PTGS is a nucleotide sequence-specific defence mechanism that can target both cellular mRNAs and viral RNAs in which a 21-25 nucleotide-long double stranded RNA (dsRNA) that is complementary to the target RNA plays a fundamental role in causing the sequence-specific degradation of RNA (Hamilton and Baulcombe, 1999). RNA silencing is a host response triggered by small interfering dsRNA that can take various forms. In higher plants, it operates as an adaptive inducible antiviral defence mechanism

(Ding, 2010; Ding and Voinnet, 2007). As a counter strategy to this, many plant viruses have evolved viral suppressors of RNA silencing (VSRs) to counteract host antiviral defence (Burgyán, 2008; Ding and Voinnet, 2007; Voinnet *et al.*, 1999). The viral suppressors of gene silencing are able to target all steps of the silencing pathway, such as viral RNA recognition, dicing, RISC assembly, RNA targeting and amplification (Burgyán and Havelda, 2011). In addition to this, viral silencing suppressors in plants play multiple key roles in virus replication, coating, movement and pathogenesis as well. Suppressor proteins encoded by cereal-infecting members of the family *Luteoviridae* were first reported by Liu *et al.* (2012). The P6 protein of BYDV-PAV mediates RNA-silencing suppression both locally and systemically. However, the mechanisms by which GAV P6 proteins interfere with the gene-silencing pathways needs further investigation.

## 2.10 Factors affecting virus infection

The rate and extent of virus infection is affected by multiple biotic and abiotic factors, which include but are not limited to: (1) virus strains/isolates and crop species/cultivars; (2) host age of infection; (3) environmental temperature; (4) existence of other species/strains of viruses and (5) plant nutrition status.

The genetic background of host plant and virus may be the most dominant factor in determining the degree of susceptibility/resistance. Symptom severity can vary greatly with BYDV isolate. An aggressive isolate of PAV (PAV-129) causes stunting and corkscrewing in otherwise PAV tolerant oat, probably because of its more diversified sequence in the polymerase gene and 3' untranslated region of the genome. Meanwhile, the location where virus particles first appear seemed to depend on the virus isolates used by Gill and Chong (1975, 1976, 1979). The severity of disease symptoms also depends on crop species and cultivar. Oats exhibit more pronounced symptoms than barley in terms of reduced tiller number, stunting, leaf chlorosis and reddening. Morphological parameters including root dry weight, root-to-shoot ratio, leaf blade area and apparent transpiration are also impacted more significantly in oats than barley (Erion and Riedell, 2012).

Early inoculation could likely increase virus concentration more rapidly. Plants inoculated at 1-2 leaf stage showed higher rate of virus multiplication than at 4-5

leaf stage, as determined by ELISA reaction result (Eweida *et al.*, 1988). The rate of virus movement varies with the developmental stage of the leaf at the time of inoculation. Movement of virus to the roots and replication occurred at a lower rate in the case of inoculation at 4-5 leaf stage than at 1-2 leaf stage (Eweida *et al.*, 1988). In oats and bread wheat, the tiller-to-tiller virus movement is less likely to occur when plants are infected at later growth stages. This was shown using the tissue-blot immunoassay method (TBIA-ELISA) (Makkouk and Comeau, 1994).

Mixed viral infections are common in cereals. These include BYDV, *wheat streak mosaic virus* (WSMV), *barley mild mosaic virus* (BaMMV), *soil-borne wheat mosaic virus* (SBWMV), *wheat spindle streak mosaic virus* (WSSMV) and *wheat dwarf virus* (WDV) (Deb and Anderson, 2008). Infection with a mix of BYDV strains of same subgroup I (PAV, MAV, and SGV serotypes) can confer cross-protection against other viruses of the same subgroup, but not against a different subgroup (Wen *et al.*, 1991). However, mixed infections with viruses from different subgroups could give more severe symptoms than infections by a single subgroup (Baltenberger *et al.*, 1987; Miller *et al.*, 1997b).

Interactions of competing species through their use of and effect on shared resources has been described in the "resource-ratio theory" (Miller TE *et al.*, 2005). In the case of interactions among the viruses that use plants as hosts, it is discovered low nutrient supply induced a competitive hierarchy between virus species (Lacroix *et al.*, 2014). At low nutrient supply rates, the co-inoculation of BYDV-PAV reduces CYDV-RPV infection rate, whereas at high nitrogen supply rate this antagonistic interaction disappears. The result suggests a competitively asymmetrical relationship where one virus species is in a superior position of utilising host resources for multiplication when the host plant grows under conditions of low nutrient supply and provides an insight into a potential novel approach to regulate virus epidemiology by controlling rates and ratios of nutrient supply.

## 2.11 Disease scoring and diagnosis

The severity of barley yellow dwarf disease is usually scored using a 0 to 5 scale (0 = no symptoms, 5 = yellowing over the whole plant) at 3–4 weeks after inoculation,

or when severe symptoms of sensitive varieties have developed (Del Blanco *et al.*, 2014). Due to environmental variation and inherent variability when using aphids for inoculation, resistance screening needs to be based on many replications to achieve reliable scores (Chéour *et al.*, 1993).

Visual assessment of symptoms is sometimes not sufficient for diagnosis of BYDV/CYDV infection since the symptoms may be confused with those caused by other biotic and abiotic stresses. Therefore, to overcome the limitations of visual diagnosis, laboratory techniques have been developed for diagnosis of the disease. Enzyme-linked immunosorbent assay (ELISA), Tissue bolting immunoassay (TBIA) and polymerase chain reaction (PCR) have been used for distinguishing the diverse virus strains (Fouly *et al.*, 1992; Habili *et al.*, 1987).

In plant virology, the enzyme-linked immunosorbent assay (ELISA) is a well-established method (Clark and Bar-Joseph, 1984). In cereal crops, BYDV infection is commonly measured by ELISA using either the double-antibody sandwich (DAS) or triple-antibody sandwich (TAS) technique (Rochow and Carmichael, 1979). The 'double antibody sandwich' enzyme linked immunosorbent assay (DAS-ELISA) introduced by Voller *et al.* (1976) has been improved by Clark and Adams (1977) for using in routine testing of plant viruses. This assay has been successfully used to detect BYDV from infected cereal (Doupnik Jr *et al.*, 1982; Lister and Rochow, 1979). ELISA is sensitive enough for detecting BYDV in leaf extracts, for simplifying survey and assay work with this phloem-limited virus (Lister and Rochow, 1979). The disadvantages of this technique are that it is time-consuming and can produce false negative results because of its detection limit of about one million virus particles per sample (Canning *et al.*, 1996). Furthermore, identifying specific BYDV/CYDV strains requires several antisera for each virus.

In contrast to ELISA, TBIA requires less time and seems to be better suited for detection of phloem limited viruses and other viruses present in low concentration in plants (Makkouk and Comeau, 1994). BYDV, a phloem-limited virus with a low concentration in plants, can be readily detected by this method. Thirteen types of viruses in *Poaceae* plants were successfully detected by TBIA and efforts were made to improve its sensitivity and versatility (Huth, 1999). The TBIA method was

first used for fresh plant material in 1990 (Lin *et al.*, 1990) and has gained popularity for many purposes, not only due to its simplicity, eliminating the need for an extraction step, but also due to its high sensitivity. A secondary labelled antibody is used in TBIA with the most common one being the goat anti-rabbit antibodies conjugated to alkaline phosphatase. Other labelled molecules such as antibodies conjugated with colloidal gold and protein A conjugated with alkaline phosphatase (Zagula *et al.*, 1990) have also been used for identifying different antigens. TBIA needs no tissue extraction and membranes can be blotted directly in the field. Membranes can be blotted in the field, stored for long periods of time before processing and transported safely across borders without risk of introducing viruses into new environments, while the blotted virus remains immunologically active for several months (Makkouk and Comeau, 1994). For the same price at least ten times more samples could be tested by TBIA than by ELISA while reusing the conjugate solution five times (Huth, 1999). The main drawback of TBIA test is qualitative, rather than quantitative.

PCR-based technologies, including quantitative real-time reverse transcription polymerase chain reaction (q-RT-PCR) are more sensitive compared to other tests, and thus, can identify a lower viral titre (Henson and French, 1993; MacKenzie *et al.*, 1997; Nassuth *et al.*, 2000). The highly sensitive technique of Real-time RT-PCR enables amplification and quantification of a precise nucleic acid sequence in real time with detection of the PCR product. It can detect and quantify from $10^2$ to $10^8$ BYDV-PAV RNA copies, which is $10^3$ times more sensitive than ELISA assays (Liu *et al.*, 2006). The RT-PCR assesses virus infection more rapidly through diagnosis. In diagnostic laboratories, the use of PCR can be limited due to its cost. Recently, more cost-effective RT-PCR assays have been developed that detect multiple pathogens simultaneously by using a multiplex of primer pairs specific to a range of different viruses that share single standard amplification conditions (Deb and Anderson, 2008).

Being both reproducible and sensitive, q-RT-PCR has been successfully used for the quantification of low levels of virus from limited tissue samples (Freeman *et al.*, 1999; Mackay *et al.*, 2002) and proved a valuable and rapid diagnostic tool (Casas *et al.*, 1999). For detecting CYDV-RPV and BYDV-PAV in cereal crops, q-RT-

PCR required only 6 and 2 h post-inoculation respectively, whereas ELISA took 10 and 4 days post-inoculation respectively (Balaji *et al.*, 2003).

## 2.12 Mapping BYDV resistance gene and their QTL

No efficient BYDV resistance has been found in the primary gene pools of bread wheat (Brettell *et al.*, 1988; Ayala *et al.*, 2001; Ayala-Navarrete and Larkin, 2011). In cultivar Anza, *Bdv1* is the only reported gene for BYDV tolerance in wheat (Singh *et al.*, 1993), but this gene, associated with a rust resistance *Lr34/Yr18* locus, does not provide actual resistance to all isolates of BYDV (Ayala-Navarrete and Larkin, 2011). Many QTL for BYDV resistance were reported from a mapping study using two populations. However, most of them showed small effect, indicating the complexity and polygenic nature of moderate BYDV tolerance in wheat (Ayala *et al.*, 2002). Among 22 QTL identified from Opata × Synthetic population and 7 QTL identified from Frontana × INIA66 population, one is located at a similar position to the *Bdv1* gene on 7DS (Ayala *et al.*, 2002)

Thus, there is a great interest in finding and utilising BYD resistance from related species. The first alien BYD resistance gene identified and of potential use in wheat, was in a disomic chromosome addition line L1, carrying a pair of *Thinopyrum intermedium* chromosomes added to wheat (Brettell *et al.*, 1988). *Th. intermedium* is, like wheat, an allohexaploid, and the resistance gene was shown to be located on the long arm of a homoeologous group 7 chromosome, namely 7JL (also called 7Ai#1L), the resistance gene was designated *Bdv2* and PCR based molecular markers developed to follow the translocation (Stoutjesdijk *et al.*, 2001). Chromosome segments were transferred from the L1 addition line to wheat through tissue culture techniques (Banks *et al.*, 1995). Eight translocation lines (TC5, TC6, TC7, TC8, TC9, TC10, TC14 and 5395) containing *Th. intermedium* translocations have been produced. Among these TC14 had the smallest translocation of *Th. intermedium* and is located in the distal 44% of the long arm of chromosome 7D (Hohmann *et al.*, 1996). Three DNA markers were used to confirm the presence of 7Ai#1 chromatin (Zhang *et al.*, 2004). The alien chromosome does not freely recombine with its wheat homoeologues, so the markers may not be physically close to the *Bdv2* gene. More recently the TC14 translocation has been recombined

with another 7DL translocation involving *Th. ponticum*, carrying the *Lr19* gene, to produce much smaller alien fragments still carrying the *Bdv2* resistance (Ayala-Navarrete *et al.*, 2007; 2013). Independently, another resistance gene, *Bdv3*, was described associated with another group 7 chromosome from *Th. intermedium* (Sharma *et al.*, 1995; Kong *et al.*, 2009)

*Th. intermedium* also carries resistance to certain strains of BYDV on a group 2 chromosome 2Ai#2 (Zhang and Klessig, 2001). Unlike the BYDV resistance gene in L1 which is located on chromosome 7Ai#1L (Brettell *et al.*, 1988), the resistance derived from a wheat-*Th. intermedium* partial amphiploid called 'Zhong5' (or Zhong4 in some literature) is on 2Ai#2 (Larkin *et al.*, 1995). This resistance gene is designated *Bdv4*. Various PCR based molecular markers have been useful for identifying 2Ai#2 (Zhang *et al.*, 1999; Lin *et al.*, 2006; Wang *et al.*, 2010). Because the group 2 alien chromosome does not recombine freely with its homoeologues in wheat, the markers developed for 2Ai#2 are not necessarily physically close to *Bdv4*.

In barley, no complete BYD resistance has been found (Ordon *et al.* 2009), but three tolerance genes (*Ryd1, Ryd2* and *Ryd3*) have been detected. *Ryd2* is located on chromosome 3HL and has been successfully incorporated in different winter and spring barley cultivars that have been grown for commercial purpose (Ordon *et al.*, 2009). Various QTL have mapped on chromosome 7H, 4H and 1H for tolerance to BYD-PAV and BYDV-RPV (Toojinda *et al.*, 2000). Two QTL were identified on chromosomes 2HL and 3HL. The one on 3HL is at a similar position to *Ryd2*. These QTL accounted for about 50% of the phenotypic variance of relative yield after BYDV infection (Ordon *et al.*, 2009). In addition, the locus *Ryd3* located on chromosome 6H explained 75% of the phenotypic variance for BYD tolerance when highly exposed to BYDV infection (Niks *et al.*, 2004).

## 2.13 Development of molecular markers for BYDV resistance

For detection of DNA polymorphism, the development and the use of molecular markers is one of the most important advances in molecular genetics. Molecular markers are effective for characterization of genetic material as they are independent of environmental conditions under which phenotypic studies are

carried out and provide a new dimension and accuracy in screening of germplasm during breeding (Tar'an *et al.*, 2005).

For the *Bdv2* resistance gene, two RAPD markers OPD04-$_{1300}$ and OPR19-$_{940}$ were identified, and one RAPD marker (OPD04-$_{1300}$) and one SSR marker (gwm37-$_{450}$) were converted into SCAR markers (Zhang *et al.*, 2002). To detect the presence of one of the group 7 *Th. intermedium* translocations (i.e. the presence of *Bdv2* gene), microsatellite marker Xgwm37 was used for selecting BYDV resistant wheat in the absence of the disease (Ayala *et al.*, 2001; Jahier *et al.*, 2009).

SCAR markers have been used to detect and to select BYD resistance genotypes in large wheat breeding programs. Compared with RAPD markers, SCAR markers are more reliable and easier to score (Paran and Michelmore, 1993). Two SCAR markers, SC-gp1 and SC-D04 have been developed to detect the presence of *Bdv2* gene in wheat (Zhang *et al.*, 2004). SCAR markers developed from the sequence identified in Stoutjesdijk *et al.* (2001) proved robust and reliable. Primers BCF5 and BCR6, were used successfully to confirm the existence of the *Bdv2* gene in the lines originating from a cross between the original source, TC14, and a Pakistani wheat variety, Inquilab91 (Kausar *et al.*, 2015).

To specifically target R-genes, an additional molecular technique known as resistance gene analog polymorphism (RGAP) is available. Resistance gene analogs (RGAs) are highly non-randomly distributed in the genome and often map to clusters which harbour major resistance genes or QTL (Hulbert *et al.*, 2001). The RGAP technique has been successfully used to develop molecular markers for identifying plant disease resistance gene (Shi *et al.*, 2001; Toojinda *et al.*, 2000, Yan *et al.*, 2003). To develop diagnostic markers for *Bdv2*, 187 primer combinations were used but among them only two RGAP markers (Tgp-$1_{350}$ and Tgp-$2_{210}$) were identified (Zhang *et al.*, 2004).

The developed express sequence tags (EST) derived markers, such as EST-RFLPs, and EST-derived PCR markers are accessible in the NCBI database. ESTs have been used for the quick development of additional molecular markers in wheat, which directly mark the coding regions of the genome. To identify specific PCR

markers for the *Bdv2* region on the basis of the homologous relationship between wheat chromosome 7D and *Th. intermedium* chromosome 7Ai#1, forty wheat EST sequences located in the distal region of 7DL were explored. 811 EST loci were mapped in wheat chromosome 7D (Qi *et al.*, 2004), and two EST-STS markers were identified for the *Bdv2* region (Ayala-Navarrete *et al.*, 2007). Several EST-based PCR markers have been developed that are associated with *Bdv2* (Scoles *et al.*, 2009). Fourteen additional EST-based PCR markers associated with the *Bdv2*-harbouring segment were developed (Gao *et al.*, 2009). These EST-PCR markers distinguish *Bdv2* from another BYDV-resistance gene located on *Th. intermedium* chromosome 2Ai-2. Markers that were used in the mapping of wheat and barley genes are listed in Table 1.

**Table 2.1** List of markers with chromosomal location used for identifying BYD resistance gene in wheat and barley

| **Marker type** | **Primer** | **Detected gene** | **Chromosome location** | **Sequence** | **Reference** |
|---|---|---|---|---|---|
| **Wheat** | | | | | |
| SCAR | BYAgi | *Bdv2* | 7Ail | F- ACT TCA TTG TTG ATC TTG CAT G<br>R-CAT GGA TAA TTC AGG GAG CAT TCT G | Stoutjesdijk *et al.* (2001) |
| SCAR | SC-gp1 | *Bdv2* | 7Ail | F-CAG GAC AAG TGA AAG CAC CTA AGC<br>R-GTC CAC AAG TCA TAT GGG GAG AC | Zhang *et al.* (2004) |
| SCAR | SC-DO4 | *Bdv2* | 7Ai1 | F- TCT GGT GAG GCA AAC CTT CTG G<br>R-TCT GGT GAG GGA GGT GTG ATG ACG | Zhang *et al.* (2004) |
| SCAR | BDF5/ BDF6 | *Bdv2* | 7Ai1 | F-TGG CAG GCT CGT CGT AC<br>R-CTG AGG TTG AGG TTG AGG C | Kausar *et al.* (2015) |
| SCAR | AD2 | *Bdv2* | 2Ai#z 7Ds.7DL-7Ai#1L | F-TGA ACC GCT TCC AGT AAT GGA C<br>R-CTG AAC CGC TTC AGC GGT TCA G | Jahier *et al.* (2009) |
| SSR | Xgwm37 | *Bdv2* | 7DL | F- ACT TCA TTG TTG ATC TTG CAT G<br>R- CGA CGA ATT CCC AGC TAA AC | Ayala *et al.* (2001) |
| SSR | Bdv3 | *Bdv2/ Bdv3* | 7E | F-CGA CGA ATT CCC AGC TAA ACT AGA CT | Kong *et al.* (2009) |

| | | | | | |
|---|---|---|---|---|---|
| | | | | R-CTT AAC TTC ATT GTT GAT CTT A | |
| RGAP | Tgp-$1_{350}$ | *Bdv2* | 7Ail | F-CGAGACGACAGGACAAGT GA<br>R- CCCCCTGGTGGTGCATC | Zhang *et al.* (2004) |
| RGAP | Tgp-$2_{210}$ | *Bdv2* | 7Ai1 | R- CCCCCTGGTGGTGCATC<br>R-CGCAAAACACCCTTGTAAA C | Zhang *et al.* (2004) |
| RGAP | OPD$04_{1300}$ | *Bdv2* | 7Ai1 | F-TCTGGTGAGG<br>R- CCTCACCAGA | Zhang *et al.* (2004) |
| RFLP | Xpsr129 | *Bdv2* | 7Ai-1 | F: CAT GGA TAA TTC AGG GAG CAT TCT G<br>R: CTG AAC ACG AAT TTG CTG AGG TTG | Gustafson *et al.* (2009) |
| EST-STS | BE404744 | *Bdv2* | 7Ai#1 | F-AGA TGG ATG GTG CCT GAC T<br>R-AAC CTC GTC TAC TGC TTC G | Gao *et al.* (2009) |
| EST-STS | BE471156 | *Bdv2* | 7Ai#1 | F: TCA CTG GTG GCC TGG ATA A<br>R: AGG TCT TGA TGT TGC GGT TCT | Gao *et al.* (2009) |
| EST-STS | BE591497 | *Bdv2* | 7Ai#1 | F: GCA CTG ATG CGA ATG AGA ACG<br>R: TGG TCA AAC CGG AAG TGG AT | Gao *et al.* (2009) |
| EST-STS | BG606695 | *Bdv2* | 7Ai#1 | F: GTT TGA TGG TAT GGC TGC TC<br>R: CCC AAA CCG AAG GAC TGT | Gao *et al.* (2009) |
| EST-STS | BQ161842 | *Bdv2* | 7Ai#1 | F: GAA TGC GTG TAA GAT GGT<br>R: GTT GTG TGG GGA GAA GAT | Gao *et al.* (2009) |
| EST-STS | BE404953 | *Bdv2* | 7Ai#1 | F: GCC TTC GAT CTC TTC GAC AC<br>R: CCC ATC TTG TCC GTC ATC AT | Gao *et al.* (2009) |
| EST-STS | BG312663 | *Bdv2* | 7Ai#1 | F: GAA GGC AGA TTA ACA GAG GC<br>R: CAA GGA CAG CGTGAA GAA G | Gao *et al.* (2009) |
| EST-STS | BE498985 | *Bdv2* | 7Ai#1 | F: GAC ATG CCG TAT TAC CAC AAG<br>R: GCC AGA GCA TCC TCC TTG | Gao *et al.* (2009) |
| **Barley** | | | | | |
| CAPS | HvYlp | *Ryd2* | 3H | F: CTC TAT GCA GCT GAA TTC TTC CCG<br>R: AGC AAA GTT CCT GCA GAT AGT TTG TG | Ford *et al.* (1998) |
| CAPS | YlpPCRM | *Ryd2* | 3H | F: AAT ACA GGA ATC TGT TGA AAG AA<br>R: TCA TCA TGG CTC GGA GAA GGT GG | Ford *et al.* (1998) |

| | | | | | |
|---|---|---|---|---|---|
| CAPS | ABG458 | *Ryd2* | 3H | F: GAG AGC CGA TGA CGG TAT GT<br>R: CTT GGA CAC ATG CCA TAT CC | Niks *et al.* (2004) |
| AFLP | YLM | *Ryd2* | 3H | F: CAG GAG CTG GTG AAA TAG TGC CT<br>R: GCT TCA CGG AGC CCT TTA A | Paltridge *et al.* (1998) |
| RFLP | Xbcd263 | *Ryd2* | 3H | F: CCG TTA CCT GCA CAA GTT GC<br>R: ACA TTT CGG TGG TGG TCT CC | Heun *et al.* (1991) |
| SSR | Bmac0018 | *Ryd3* | 6H | F: GTC CTT TAC GCA TGA ACC GT<br>R: ACA TAC GCC AGA CTC GTG TG | Niks *et al.* (2004) |
| SSR | HVM14 | *Ryd3* | 6H | F: AAC TCT TCG GGT TCA ACC AAT A<br>R: CGA TCA AGG ACA TTT GGG TAA T | Niks *et al.* (2004) |
| SSR | HVM65 | *Ryd3* | 6H | F: AGA CAT CCA AAA AAT GAA CCA<br>R: TGG TAA CTT GTC CCC CAA AG | Niks *et al.* (2004) |
| SSR | HVM74 | *Ryd3* | 6H | F: AGG AAG TCA TTG CGT GAG<br>R: TGA TCA AGA ATG ATA ACA TGG | Niks *et al.* (2004) |
| SSR | HVM22 | *Ryd3* | 6H | F: TTTTGGGGGATGCCTACATA<br>R: TTTCAAATGGTTGGATTGGA | Niks *et al.* (2004) |

## 2.14 Transgenic resistance to BYDV

Sources of natural resistance to BYDV and CYDV are few (Jin *et al.*, 1998), therefore researchers have explored whether genetic-engineering could potentially provide forms of synthetic resistance for effective and sustainable crop protection. Transgenic virus-resistant plants were first produced in 1986 by genetically engineering tobacco plants to express the coat protein of tobacco mosaic virus resulting in a delay of viral symptoms (Abel *et al.*, 1986). The coat protein approach has been successfully applied in many dicotyledonous host/virus systems (Wilson, 1993), including potato leaf roll *luteovirus* (PLRV) in potato (Barker *et al.*, 1992). The most celebrated success was the release in 1998 of papaya ring spot virus (PRSV)-resistant papaya in Hawaii using the coat protein gene, credited with saving the industry. McGrath *et al.* (1997) transformed barley and oat with modified coat

protein genes from BYDV-MAV (oats), CYDV-RPV (oats) and BYDV-PAV (both oats and barley), achieving only low levels of resistance with inconsistent inheritance.

Expressing viral genes, other than the coat protein gene, can sometimes also confer a degree of resistance. Koev *et al.* (1998) transformed oat plants with a construct consisting of the CaMV 35S promoter driving the 5' half of the BYDV-PAV genome, which includes the RNA-dependent RNA polymerase gene. T2 and T3 generation plants inoculated with BYDV developed disease symptoms initially, but recovered and were fertile.

Virus immunity through Post-Transcriptional Gene Silencing (PTGS) can be induced in plants using transgenes that encode double stranded or self-complementary "hairpin" (hp) RNA (Eamens *et al.*, 2008; Smith *et al.*, 2000). Wang *et al.* (2000) made the first attempt to apply RNA silencing for BYDV protection in barley. Barley lines with complete immunity to BYDV were successfully generated by using a short hairpin RNA-encoding construct (shRNA or hpRNA) driven by the maize (*Zea mays*) ubiquitin promoter and targeting the 5' end of the BYDV-PAV genome. Presumably the double-stranded RNA induces the host's post- transcriptional gene silencing system (PTGS) (Waterhouse *et al.*, 2001). Similar constructs were employed by the same authors to achieve some resistance in wheat (Abbott *et al.*, 2002).

Abbott *et al.* (2002) reported that in field conditions, BYDV-PAV is sometimes associated with the related luteovirus, CYDV-RPV. When the transgenic plants with the hpRNA encoding BYDV-PAV sequence were challenged with BYDV-PAV and CYDV together, the plants were susceptible to CYDV but immune to BYDV-PAV. This showed that the immunity is sequence specific and not broken down by the presence of CYDV. It also suggests that the immunity will be robust in the field and very useful in minimizing losses in barley production due to BYDV-PAV.

These initial successes with hpRNA induced resistance in cereals, have been followed up by independent studies. Transgenic wheat expressing hpRNAs of

sequences encoding the replicase of BYDV (GPV strain), with an antisense RNA loop of part of the capsid protein gene, showed a high level of resistance in 28.5% of 21 lines, while the other lines showed no symptoms when infected by the virus at a lower dose (Yan *et al.,* 2007). Transgenic wheat expressing hpRNAs against the polymerase gene of BYDV (PAV) did not show symptoms after inoculation with the virus (Yassaie *et al.,* 2011). Both shRNA and artificial microRNA (amiRNA) approaches have been refined to confer immunity in wheat against Wheat Streak Mosaic Virus (Fahim *et al.,* 2010; 2012).

## 2.15 Methods of controlling BYDV: breeding strategies

Chemical control of aphids and the use of resistant varieties are the two main control strategies for BYDV infection. Chemical control is based on insecticides targeting the aphid vectors of the virus, but this method is costly and environmentally damaging (Chain *et al.*, 2005). Continuous use of pesticide poses potential health hazards and impact on wildlife and ecosystems (Power, 2010; Stoate *et al.*, 2001). Therefore, breeding resistant/tolerance varieties is the most effective and economical method for controlling BYDV (Ordon *et al.*, 2004).

The *Ryd1* recessive gene was identified in the barley cultivar Rojo (Suneson, 1955), but this gene provided very little resistance, and is rarely employed in breeding programmes. BYD-resistant genes $Ryd_2$ and *Ryd3* were identified in Ethiopian barley landraces (Niks *et al.,* 2004; Schaller *et al.,* 1964). $Ryd_2$ is the most significant BYD resistant gene in barley and is widely used in breeding programs. This gene has provided substantial levels of resistance against BYDV for many years in a range of barley cultivars and continues to be used by breeders (Burnett *et al.*, 1995; Beoni *et al.*, 2016). Combining $Ryd_2$ and *Ryd3* produces a high degree of protection in barley than either gene alone (Riedel *et al.*, 2011).

The *Bdv1* gene was detected in the North American bread wheat cultivar Anza (Singh *et al.*, 1993). Because *Bdv1* is linked to the highly desirable adult rust resistance Lr34/Yr18 locus, it has been widely deployed but delivers very little protection to BYDV (Ayala-Navarrete and Larkin, 2011). The *Bdv2* gene originated from intermediate wheatgrass, *Thinopyrum intermedium,* and has been introduced into some wheat cultivars as one of the group 7 translocation lines, either TC6 or

TC14 (Stoutjesdijk *et al.*, 2001). At least four wheat varieties were developed deploying *Bdv2* in Australia and China. The cultivars Mackellar (derived from TC14) and Glover and Manning (derived from TC6) were released in Australia, and cultivar Lingkang (derived from an independent translocation, Yw443) was released in China (Ayala-Navarrete *et al.*, 2007).

## 2.16 Conclusion

BYDV can lead to phloem degradation and collapse of sieve elements, resulting in a reduction in plant growth (dwarf appearance) and colour changes of leaf blades along the vascular bundles, especially of leaf tips. BYDV also induces significant changes of host plant biology at microscopic and molecular levels, including ultrastructural alterations, changes of chemical compounds content and regulation of photosynthesis and respiration rate. The observed host alterations not only represent plant responses to BYDV invasion, but are responsible for constructing a defending wall of resistance possibly by blocking the channel of virus spread (callose deposition) and inducing oxidative burst. The hypersensitive reaction (HR) response initiated by Avr/R protein interaction leads to metabolic changes in defence hormones, accumulation of reactive oxygen species (ROS) and alteration of membrane permeability. Viral movement protein (MP) encoded by plant virus genomes plays a key role in virus spread *in vivo* and systemic infection. The MPs of some virus species are essential in cell-to-cell and systemic viral transport. However, the role of BYDV MP in inducing symptoms and intracellular and intercellular transport of the viral genome still requires thorough investigation.

Development of durable resistant varieties to viral diseases in general has proved economical and environmentally sustainable. Experience with BYDV resistances is more limited but also looking positive. The availability of different molecular tools allows characterization of genes of interest and identification of plants carrying the target genes and might well serve to improve the efficiency of conventional breeding. Molecular dissection should now enable more rapid identification of BYDV resistance genes and linked QTL. Genomic selection will also enable varieties to be developed with effective combinations of multiple loci with major and minor effects, hopefully achieving greater resistance and more

durable resistance. A major advantage of using markers for resistance to difficult diseases such as BYD is that it avoids the difficulties of managing the viruliferous aphids and having disease development confounded by environmental factors. The usefulness of alien sources, from the secondary or tertiary gene pools, has been demonstrated, such as the *Bdv2* resistance from *Th. intermedium* deployed in wheat. Perennial grasses have proved a particularly rich source of virus resistances. The search needs to continue to find new sources of resistance for cereal breeding programs to strengthen and broaden the defences against this widespread and damaging disease.

# Chapter 3. A screening method to detect BYDV-PAV resistance in cereals under glasshouse conditions[2]


## Abstract

A reliable method was developed to screen cereal crops for BYDV-PAV resistance in glasshouse experiments. This also entailed the evaluation of traits associated with Barley yellow dwarf virus (BYDV) infection such as leaf discolouration, reduction in growth, biomass and yield traits, and percentage of virus-infected plants, using enzyme-linked immunosorbent assay (ELISA) and tissue blot immunoassay (TBIA). Four glasshouse experiments were conducted with eight wheat, barley and oat varieties mechanically inoculated with BYDV-PAV at the 2-leaf stage, using different numbers of viruliferous aphids and different inoculation periods and temperatures. Inoculation with 5–10 viruliferous aphids per plant for 4 days led to a high percentage of infection in susceptible varieties, indicating that this is an effective BYDV screening method when selecting for resistance in cereal crops. For barley and oat, visual evaluation of symptoms is considered adequate for assessing BYDV resistance. However, for wheat it is necessary to evaluate BYDV resistance by ELISA/TBIA tests and plant biomass (at early stage) and grain number and yield (at late stage) measurements.


## 3.1 Introduction

Barley yellow dwarf (BYD) is one of the most widespread and serious viral diseases in the world. The causal agent, *Barley yellow dwarf virus* (BYDV), has a large host range, infecting many grass species in the family Poaceae, and is responsible for serious yield losses in barley, wheat and oat (Halbert and Voegtlin, 1995).

Barley yellow dwarf has a negative effect on plant growth and development as it decreases shoot biomass, diminishes plant vigour and delays heading date. Symptoms of BYDV infection include leaf discolouration (reddening in oat, yellowing in barley, and either reddening or yellowing in wheat) and stunting (Kosova *et al.*, 2008). The foliar symptoms start from the leaf tip and spread toward

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the leaf base, particularly on the flag leaves. The disease leads to yield reduction by impacting yield components including the number of heads per plant, grains per head and grain weight (McKirdy *et al.*, 2002). Consequently, yield losses can range from 5% to 80% (Perry *et al.*, 2000). Furthermore, affected plants are more prone to fungal infection and abiotic stresses (D'Arcy, 1995). Tolerant plants present attenuated symptoms and lower yield losses even when viruses multiply actively *in vivo*. In resistant plants, virus multiplication and spread are inhibited or reduced, and disease symptoms are usually highly localized or not even visible (Kang *et al.*, 2005).

The BYDV strains vary strongly in their symptom manifestation, vector transmission efficiency, host preferences and their serological and molecular properties. Among them, BYDV-PAV is the most prevalent and damaging. BYDV-PAV is transmitted obligatorily by the aphid species *Rhopalosiphum padi* and *Sitobion avenae* (Miller and Rasochová, 1997). The phloem-restricted virus is transmitted to plants when viruliferous aphids inject saliva through the stylet during feeding (Ng and Perry, 2004).

The severity of the disease largely depends on the inoculation time. In general, plants are most vulnerable to the virus when they are infected at an early growth stage. Inoculation at the 2- to 3-leaf stage of plant development was the most effective stage to evaluate BYDV tolerance (Goulart *et al.*, 1989). The transmission of BYDV is also influenced by the duration of the virus access periods and the availability of virus in the source plants (Gray *et al.*, 1991).

The leaf symptoms commonly found in BYDV-infected plants are often used to select plants for BYD resistance, with the development of many resistant varieties of wheat, barley and oat being based on this method (Kosova *et al.*, 2008; Veškrna *et al.*, 2009). However, some other biotic or abiotic stresses may also cause similar symptoms, which makes it difficult to score for BYDV resistance based on symptoms alone. To overcome the limitation of visual diagnosis, tissue blot immunoassay (TBIA) and enzyme-linked immunosorbent assay (ELISA) have been used for detecting virus strains and their multiplication. ELISA has proven to be an efficient means of assessing BYDV resistance even with a low infection rate at an early plant growth stage (Chéour *et al.*, 1993).

Breeding of resistant or tolerant varieties is the most effective and economical method for controlling BYDV (Ordon *et al.*, 2004). For reliable selection of BYD tolerant varieties, uniform inoculation is essential and requires the controlled application of reared viruliferous aphids. Natural infections in the field can be too unreliable for effective breeding progress. Controlled application of viruliferous aphids also enables the assessment of particular virus isolates and impacts of inoculation at various developmental stages. To introduce BYDV resistance into agronomically valuable but BYDV susceptible barley and wheat germplasm, it is important to develop methods that enable a rapid and reliable detection of the presence of resistance genes in the germplasm and determine the best selection criteria that contribute to yield reduction due to BYDV infection.

The objectives of this study were to compare the effect of BYDV infection on plant growth and yield characteristics of wheat, barley and oat varieties differing in their BYDV-PAV resistance level, and to evaluate the effectiveness of different screening methods.

## 3.2 Materials and Methods

Four glasshouse pot trials assessed the effect of BYDV-PAV infection on wheat, barley and oat varieties with differing levels of resistance and tolerance. Experiment I was conducted in Armidale, NSW, and experiments II, III and IV were conducted at Mount Pleasant Laboratories, Prospect, Tasmania, Australia. Five wheat varieties, Mace, Revenue, Mackellar, and either XuBYDV (experiment I) or Manning (experiment II, III and IV), two barley varieties (Franklin and Flagship) and two oat varieties (Eurabbie and Bass) were grown to assess varieties with varying levels of resistance/tolerance to BYDV (Table 3.1). As experiment I showed no obvious visual symptoms due to fewer aphids being inoculated (three aphids per plant) and a shorter inoculation time (2 days), experiment II was conducted with a higher number of aphids (five aphids per plant) and a longer inoculation time (4 days), which led to better visual symptoms in susceptible varieties. Experiment II was then repeated (experiment III) under a different temperature profile with an increased number of aphids (10 aphids per plant). Results from experiments II and III were validated in experiment IV, which compared the effectiveness of different screening methods under the same environmental conditions.

### 3.2.1 Aphids

Colonies of the bird cherry oat aphid, *R padi*, were established by collecting adult aphids from field populations, and transferring newly laid juveniles to uninfected glasshouse-grown plants before they fed. The populations were then reared on oats (cv. Coast Black) in a cage under natural light and 25/15 °C day/night temperatures (experiment I), or on barley (cv. TAM407227) in a controlled environment room at 20 °C and a 14 h light/10 h dark cycle (experiments II, III and IV).

### 3.2.2 Virus isolate

The BYDV-PAV isolate was collected from a wheat field in northern NSW in 2010 and maintained in oat plants (cv. Coast Black) in an aphid-free glasshouse, with groups of tillers being vegetatively propagated as required to maintain active plant growth. Infection of the plants was confirmed by regular TBIA testing of the tillers. The virus isolate was transferred and maintained in barley plants (cv. TAM407227, a BYDV-susceptible genotype) in preparation for experiments II, III and IV, with transmission to new plants every 6 weeks using aphids contained in clip cages (Fig. 3.1). Clip cages were removed after 2 days to allow aphid populations to grow and spread.

### 3.2.3 Experimental design

All experiments were factorial designs, with eight varieties and two treatments (BYDV-PAV and controls). Experiment I had four replicates arranged in a blocked design, while experiments II, III and IV had three randomly arranged replicates.

### 3.2.4 Growing conditions

All four experiments were carried out in glasshouses, with plants growing under natural lighting conditions, with different day/night temperatures (Table 3.2). Eight seeds of each variety were sown into 15 cm pots filled with a 2:1 ratio of potting mix and sand, before being thinned to five plants after emergence. Pots were watered as required until the plants reached maturity and fertilized every 2 weeks with a soluble fertilizer (Thrive; Yates) at the rate of 80 mg per pot.

The BYDV-PAV inoculation was performed at the 2-leaf stage, with viruliferous wingless aphids being transferred to the second leaf of each plant, contained in clip

cages that allowed feeding on the leaf blade. In different experiments, different numbers of aphids were allowed to feed on each plant for different days (Table 3.2). At the end of the feeding period, aphids were either physically removed, or sprayed with lambda-cyhalothrin (Karate; Syngenta).

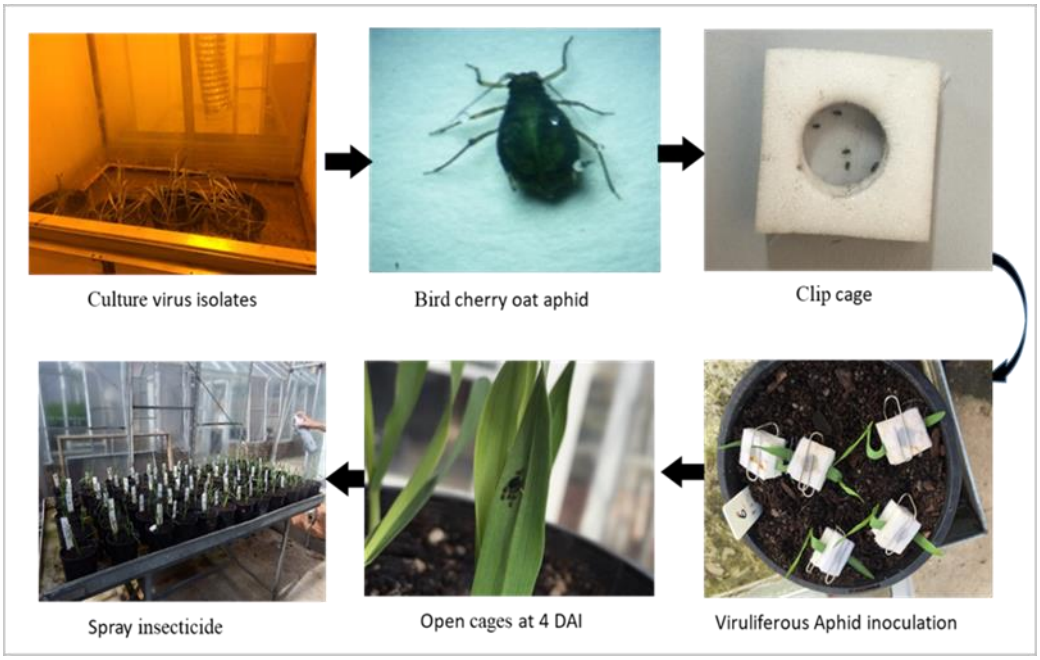



**Fig. 3.1** Inoculation method for detecting BYDV infection in cereal crops

### 3.2.5 Assessment of infection rate

The virus status of the individual plants was assessed by TBIA (experiment I) or ELISA (experiments II and III) at approximately 6 weeks after infection (WAI). TBIA testing followed the method outlined in Schwinghamer *et al.* (2014). One tiller was removed from each plant, and fresh cross-sections of the tiller were blotted onto nitrocellulose membranes before being assayed with polyclonal (BYDV-PAV/MAV) and monoclonal (BYDV-PAV) antisera (Agdia) and visually assessed under a dissecting microscope.

ELISA testing used polyclonal BYDV-PAV antibodies and followed the method of Clark and Adams (1977). Results were assessed by using a Multiskan RC plate reader with GENESIS software (Lab Systems). All samples were tested twice. Samples with absorbance values greater than twice the mean of negative controls in one or both samples were considered positive.

**Table 3.1** Reaction to Barley yellow dwarf virus (BYDV) disease of selected cereal varieties.

| Crop Species | varieties | Growing season | Status | Reference or comment |
|---|---|---|---|---|
| *Triticum aestivum* | Mackellar | Winter | Resistant (*Bdv2* gene) | Banks *et al.* (1995b) Ayala-Navarrete *et al.* (2007) |
| | Manning | Winter | Resistant (*Bdv2* gene) | Banks *et al.* (1995b) |
| | XuBYDV | Winter | Resistant | Chinese breeding line |
| | Mace | Spring | Susceptible | Zhou *et al.* (2015) |
| | Revenue | Winter | Susceptible | Zhou *et al.* (2015) |
| *Hordeum vulgare* | Franklin | Spring | Resistant (*Ryd2* gene) | Rasmusson and Schaller (1959) Raman and Read (1999) |
| | Flagship | Spring | Susceptible | Wallwork *et al.* (2009) |
| *Avena sativa* | Bass | Spring | Tolerant | Schaefer (2017) |
| | Eurabbie | Spring | Susceptible | Wallwork *et al.* (2009) |

**Table 3.2** Selected cereal varieties inoculated with BYDV-PAV at the two-leaf stage using different aphid numbers and inoculation periods

| Experiment | Aphids per plant | Inoculation access period (days) | Average Temperature (°C) | | Location |
|---|---|---|---|---|---|
| | | | Day | Night | |
| I | 3 | 2 | 24 | 15 | Armidale, NSW |
| II | 5 | 4 | 19 | 12 | Mount Pleasant Laboratories, TAS |
| III | 10 | 4 | 24 | 15 | Mount Pleasant Laboratories, TAS |
| IV | 3, 5 and 10 | 2, 4 and 4 respectively | 28 | 15 | Mt. Pleasant Laboratories, TAS |

### 3.2.6 Visual Assessment infection

Disease severity (percentage of leaf tissue with symptoms) was assessed on all plants in each pot at 6 WAI and again at head emergence stage in experiments II and III. However, in experiment IV, disease severity was assessed at 4 WAI. All plants in experiment II, and all winter wheat plants in experiments III and IV were evaluated at the tillering stage. Barley, oat and spring wheat plants in experiments III and IV were evaluated at the stem elongation stage. The severity of symptom

development was based on the proportion of leaves showing red to yellow discolouration in the inoculated plants and was scored on a 0–5 scale, where 0 = whole plant without symptoms; 1 = few leaves showing discolouration; 2 = approximately 20% of leaves affected; 3 = 40% of leaves affected; 4 = 60% of leaves affected; and 5 = almost all the plant affected (Suppl. Fig. 3.1). Average visual symptom scores were calculated.

### 3.2.7 Agronomic traits

Plant height was recorded at harvest in experiment I, at 6 WAI in experiments II and III and at 4 WAI in experiment IV. Plant height was measured from the top rim of the pot to the top of the main tiller. At maturity, all plants were harvested in experiment I, while four of the five plants per pot were harvested in experiments II and III. Plants were dried for 2 days at 30 °C before the shoot biomass, grain yield and number of effective tillers were recorded in experiments I–III. Total grain number and thousand-kernel weight were also recorded in experiments II and III. The grain yield components of Revenue, Mackellar and Manning were not measured in experiment III as all three varieties are winter types and did not vernalize in this trial. In experiment IV, only growth parameters (plant height, tiller number and shoot fresh and dry weight) were measured in each variety at 4 WAI.

### 3.2.8 Data analysis

Yield data were analysed in R v. 3.0.1 as linear mixed effects models using the packages stat, NLME and LSMEA (R Core Team, 2016). Analysed models were factorial designs, with variance structures included where relevant. Tiller counts were analysed with a gamma distribution. Infection rates were analysed using a generalized linear model with a binomial distribution. Models were simplified using Akaike information criterion (AIC) values as criteria to remove nonsignificant terms, and residuals were checked after analysis to validate the models used. Effects were considered significant when $P < 0.05$. Comparisons between treatments were made using Tukey's HSD. Results for experiment I were initially analysed with all plants included, but infection rates were low in some varieties, and the reduced growth in infected plants was hidden by the larger number of uninfected plants. The data were then re-analysed using only plants confirmed to be infected by TBIA in

the BYDV-PAV treatments, and only in the varieties where there were sufficient numbers of infected plants to allow a valid comparison.

## 3.3 Results

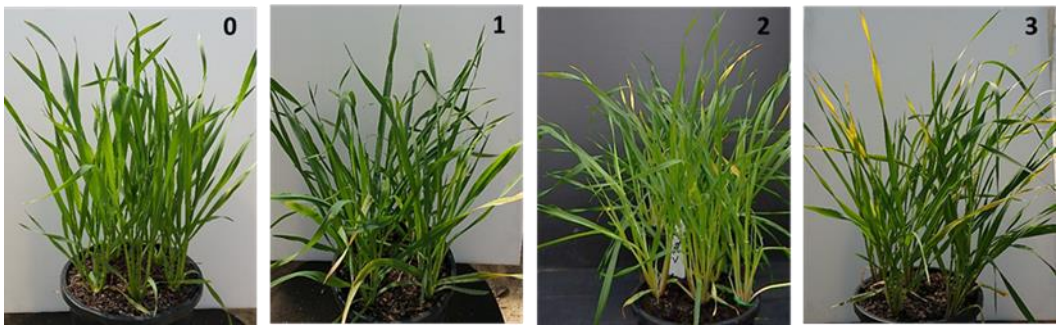
### 3.3.1 Disease incidence and severity

Barley yellow dwarf infection of the plants by aphids was confirmed, with overall infection rates increasing with more exposure to viruliferous aphids between experiments I and III, while susceptible varieties generally had significantly higher infection rates than resistant varieties across the trials (Fig. 3.2a, c). In experiment I, infection rates of the susceptible varieties ranged from 31% to 55% for Bass and Mace, while in the resistant varieties, Franklin had no infection and Mackellar and XuBYDV both had 3.7%. The susceptible barley variety Flagship also had only 3.7% infection, but this low level did not re-occur in the other experiments (Fig. 3.2b). While there was a considerable variation in infection rates, differences were only significant between Mace (55%) and the varieties with 3.7% infection rate or less. Infection rates in experiment II were 0% and 6% in the resistant wheat varieties Mackellar and Manning, and 60% in the resistant barley Franklin, while the susceptible lines had infection rates from 86% to 94%. Infection rates were higher again in experiment III, with the resistant Mackellar, Manning and Franklin having 17%, 25% and 92% infection respectively, while the susceptible lines were all 100% infected.

Symptom expression was recorded at 6 WAI and at head emergence stage in experiments II and III and at 4 WAI in experiment IV. In these experiments, four wheat varieties (Mackellar, Manning, Mace and Revenue) did not show any visual symptoms up to 6 WAI. However, at spike initiation stage, average visual symptom score (VSS) values ranged from 0 to 1.66 in both experiments II and III. At head emergence stage, leaf discolouration decreased in barley varieties and increased in oats.

In experiment IV, the responses of all varieties to virus inoculation were similar to that in experiments II and III. Almost all infected barley and oat varieties developed visual symptoms independent of the aphid numbers (3/5/10 aphids per plant) for inoculation, except for resistant barley variety Franklin, which did not show any

leaf discolouration when using three aphids for 2 days. Disease severity was more prominent when using higher numbers of viruliferous aphids, with 10 aphids per plant causing the highest leaf discolouration of both susceptible barley variety Flagship (3.08) and susceptible oat variety Eurabbie (2.8). In contrast, wheat varieties did not show any visual symptom at 4 WAI (Fig. 3.2d).




**Suppl. Fig. 3.1** BYD symptom severity scores (Score 0= whole plant without symptoms; score1= few leaves showing discoloration, score 2 = about 20% leaf of the plant consisting of discoloration; score 3= 40% leaf of the plants showing yellowing).

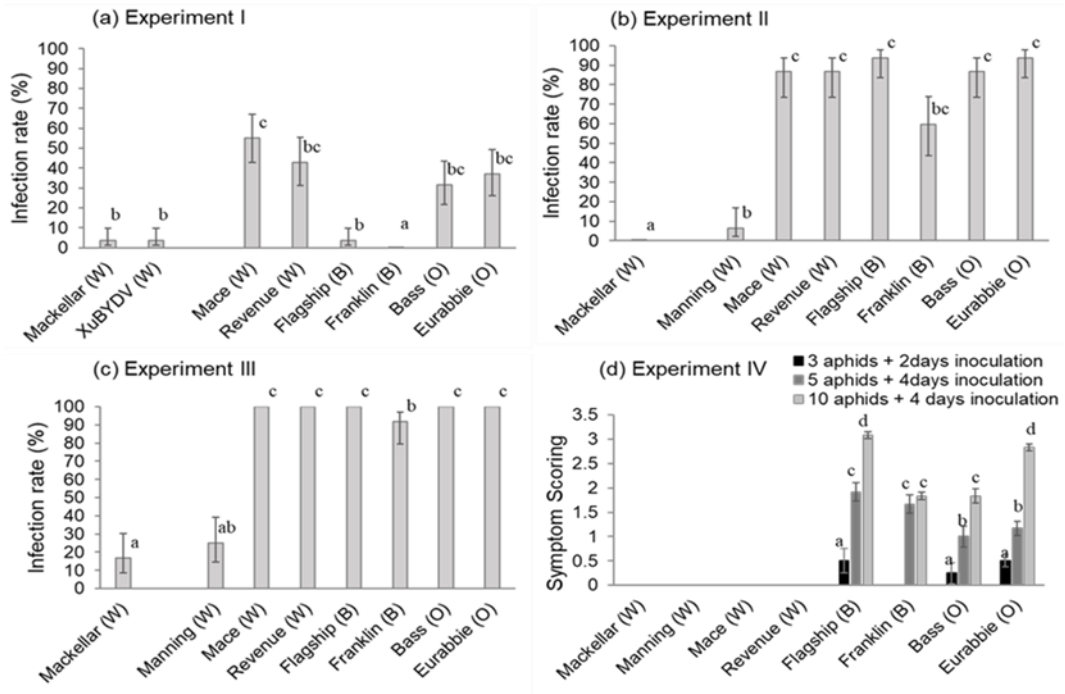



**Fig. 3.2** Infection rates (%) (a, b, c) and symptom scoring (d) in the BYDV-PAV treatment for wheat (W), barley (B) and oat (O) varieties. (a) Three aphids + 2 days inoculation, (b) five aphids + 4 days inoculation, (c) 10 aphids + 4 days inoculation. Standard error bars are shown on each column. Letters over a column indicate significance of differences between varieties and treatments.

### 3.3.2 Plant biomass

Only the susceptible wheat varieties Mace and Revenue had significant reductions in biomass in the BYDV-PAV treatment when all plants were included (Fig. 3.3a). However, when only the infected plants in the PAV treatments were compared to the controls, then significant biomass reductions occurred in all susceptible wheat and oat varieties (Fig. 3.3b). In experiments II and III (Fig. 3.3c, d), infection with BYD resulted in significant reductions in plant biomass in all of the susceptible varieties.

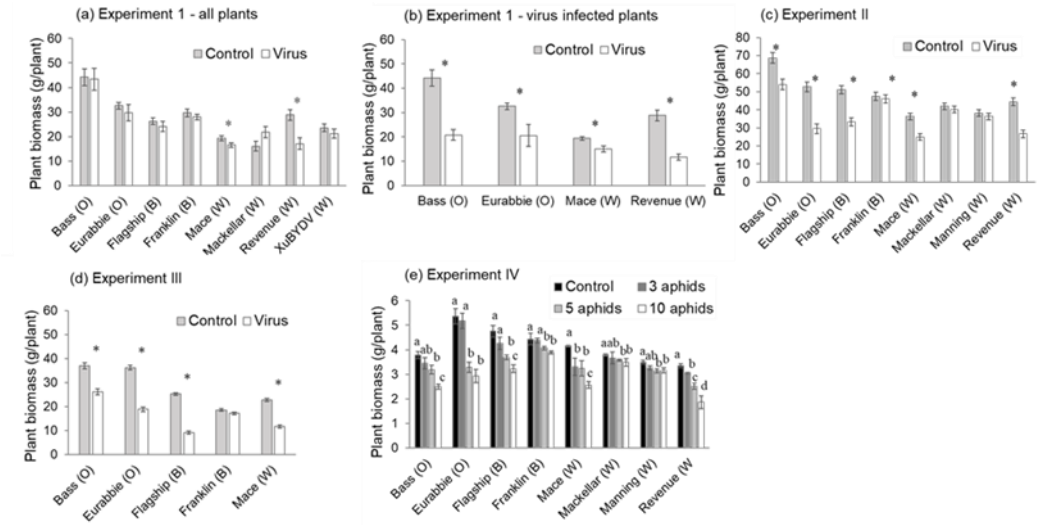



**Fig. 3.3** Average biomass weight (g) in the BYDV-PAV treatment and control plants of wheat (W), barley (B) and oat (O) varieties. (a, b) Three aphids + 2 days inoculation; (c) five aphids + 4 days inoculation; (d) 10 aphids + 4 days inoculation; (e) 3, 5, 10 aphids + 2, 4, 4 days inoculation. Standard error bars are shown on each column. *and letter indicates a significant difference in biomass between the control and virus infected treatments for that variety.

Plant biomass (straw plus grain DW) ranged from 19.34 to 44.20 g in experiment I, 36.37 to 68.66 g in experiment II, and 18.54 to 36.87 g in experiment III among the genotypes under control conditions (Fig. 3.3). However, in the PAV treatment, plant biomass ranged in all genotypes from 11.71 to 20.78 g in experiment I, 24.91 to 54.00 g in experiment II and 9.04 to 26.16 g in experiment III. Losses in susceptible varieties varied between experiments, but were generally between 30% and 50%, with wheat cv. Mace ranging from 22% to 49%, and Revenue from 40% to 59%, and susceptible oat cv. Eurabbie from 37% to 48%, and tolerant oat cv.

Bass from 21% to 53%. In contrast, there was no difference in biomass in any of the three resistant varieties (Mackellar, Manning and Franklin).

In experiment IV, significant differences in biomass (shoot DW) were found in BYDV inoculated and non-inoculated control plants for all susceptible varieties when inoculated with higher numbers of aphids, i.e. five and 10 aphids per plant. The highest reduction of biomass (dry weight) occurred in plants inoculated with 10 aphids. The reduction rates of dry weight varied within variety, with the lowest reduction occurring in the resistant wheat cv. Mackellar (7%) and the highest in susceptible oat cv. Eurabbie and wheat cv. Revenue 44% (Fig. 3.3e). The same trends were shown in fresh weight (data not shown).

### 3.3.3 Plant height

In experiment I, there was no significant effect of the virus treatment when all plants in the treatments were analysed (Fig. 3.4a). However, when only the infected plants in the BYDV-PAV treatment were included (Fig. 3.4b), there was a significant ($P < 0.001$) reduction in plant height of only in the susceptible wheat cv. Revenue.

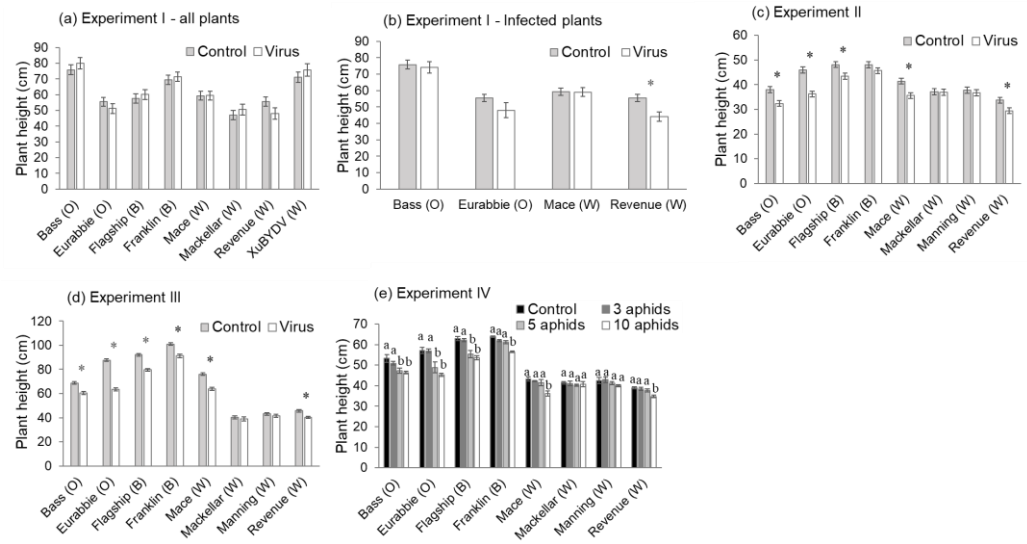



**Fig. 3.4** Average biomass weight (g) in the BYDV-PAV treatment and control plants of wheat (W), barley (B) and oat (O) varieties. (a, b) Three aphids + 2 days inoculation; (c) five aphids + 4 days inoculation; (d) 10 aphids + 4 days inoculation; (e) 3, 5, 10 aphids + 2, 4, 4 days inoculation. Standard error bars are shown on each column. *and letter indicates a significant difference in biomass between the control and virus infected treatments for that variety.

In both experiments II and III, the BYD treatment caused significant ($P < 0.001$) decreases in plant height at 6 WAI compared to control plants in most varieties (Fig. 3.4c, d). The only exceptions were the resistant wheat cvs Mackellar and Manning, with no significant difference between infected and control treatments, and the resistant barley cv. Franklin, which was not significantly affected in experiment II. Plant height ranged from 35.6 to 63.8 cm, 29.4 to 40.2 cm and 43.4 to 79.7 cm for BYDV-PAV inoculated plants of the susceptible cvs Mace, Revenue and Flagship, respectively, while values ranged from 41.4 to 76.1 cm, 33.7 to 45.7 cm and 48.1 to 92.0 cm for the control plants of the above varieties, respectively. The resistant Franklin had a mean value of 91.2 cm in virus-inoculated plants compared to 101.0 cm for the control plant in experiment III.

### 3.3.4 Tiller number

The number of effective tillers was reduced by BYDV-PAV infection, but to a lesser extent than other yield parameters. In the susceptible wheat varieties, inoculation with BYDV-PAV caused a significant reduction in effective tiller number. In virus-infected Mace, tiller number ranged from 7 to 8.3 compared to 9.3 to 10.2 for the control in experiments II and III, but no significant change was observed in experiment I. Revenue showed the greatest reduction in tiller number, with an average value of 2.1 and 9.0 in experiments I and II, respectively. The resistant wheat varieties showed no significant changes except for Mackellar in experiment I, where tiller number increased in BYDV-PAV-inoculated plants. The barley varieties were largely unaffected except for Flagship in experiment I, where the average number of tillers was 11.0 in BYDV-PAV-inoculated plants while in control was 14.8, although infection rates were only 5%. Eurabbie oats had a significant reduction in tiller numbers in experiments II and III (Fig. 3.5b, c), but had no significant change in experiment I in either analysis method, while the tolerant Bass showed a significant reduction in tiller number, with an average value of 9.7 compared to 11.7 in the control, in experiment III only. Tiller numbers of susceptible varieties were also significantly affected by virus infection when using higher numbers of aphids (5–10 aphids per plant) in experiments IV (Fig. 3 5d).

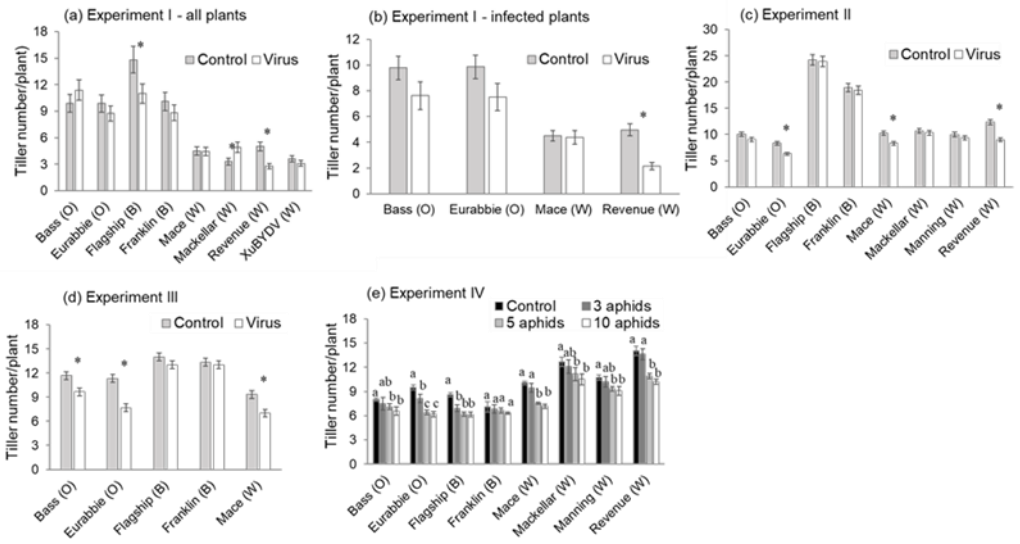



**Fig. 3.5** Average number of tillers in the BYDV-PAV treatment and control plants of wheat (W), barley (B) and oat (O) varieties. (a, b) Three aphids + 2 days inoculation; (c) five aphids + 4 days inoculation; (d) 10 aphids + 4 days inoculation; (e) 3, 5, 10 aphids + 2, 4, 4 days inoculation. Standard error bars are shown on each column. * and letter indicates a significant difference in tiller number between the control and virus infected treatments for that variety.

### 3.3.5 Effect of BYDV infection on thousand-kernel weight (TKW), number of grains per plant and grain weight per plant

The TKW from plants in the BYDV-PAV treatment was not significantly reduced by virus inoculation in resistant wheat and barley varieties in experiments II and III (Fig. 3.6a, b). The reductions in TKW in the inoculated susceptible oat, wheat and barley genotypes were significant. The virus tolerant oat cv. Bass had a significant reduction in TKW (40 g) compared to control (43 g) in experiment II only. The number of grains per plant was significantly reduced in all susceptible varieties in both experiments, but not in the resistant wheat and barley varieties (Fig. 3.6c, d).

The grain yield was significantly different in BYDV-PAV-inoculated susceptible varieties compared to control across the three experiments I, II and III, while the resistant varieties had no significant reduction. The percentages of grain yield loss due to BYDV infection were significantly different ($P < 0.05$) between most of the susceptible varieties. In both experiments II and III, the highest percentage of yield loss was recorded on Flagship, followed by Eurabbie, Revenue and Mace. The lowest yield loss was recorded on Franklin. In plants inoculated with BYDV-PAV,

the decrease in grain yield was up to 58% in experiment II and 59% in experiment III (Fig. 3.7). The resistant wheat cvs Mackellar and Manning and barley cv. Franklin showed no significant reduction in grain yield.

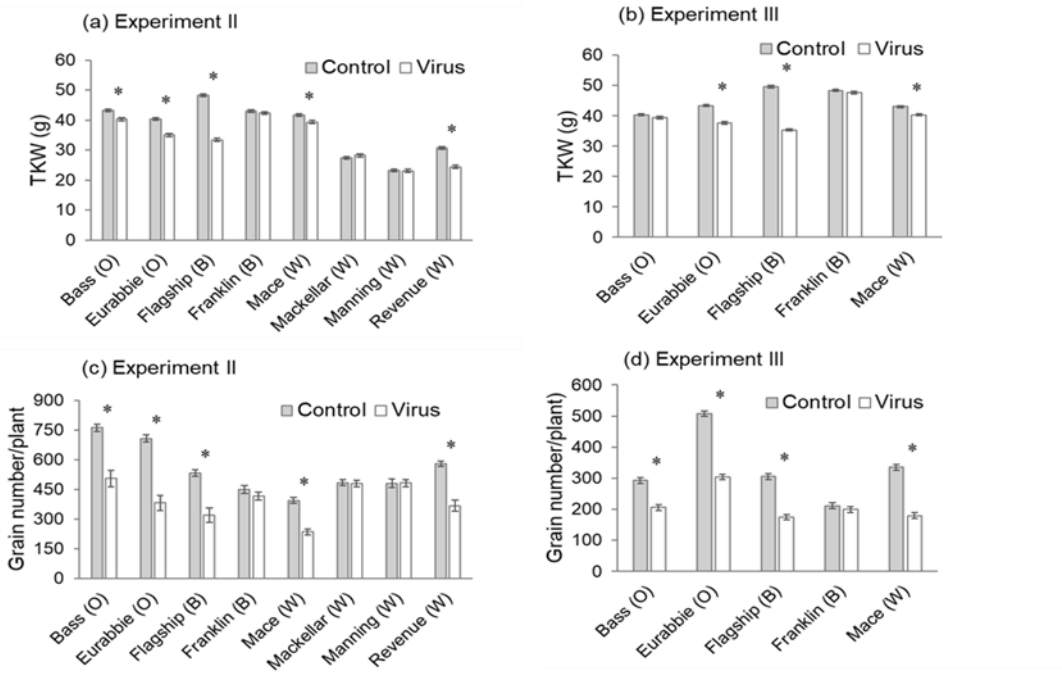



**Fig. 3.6** Thousand-kernel weight (g) (a, b) and grain number per plant (c, d) in the BYDV-PAV treatment and control plants of wheat (W), barley (B) and oat (O) varieties. (a, c) Five aphids + 4 days inoculation; (b, d) 10 aphids + 4 days inoculation. Standard error bars are shown on each column. * indicates a significant difference between the control and virus infected treatments for that variety.

## 3.4 Discussion

In this study, experiments were conducted to investigate the screening technique for BYDV resistance to detect disease resistant varieties. The study showed that the proportion of infected plants depended on plant genetic background, and a combination of number of viruliferous aphids and inoculation access periods. In experiment I, each plant was inoculated with three viruliferous aphids for 2 days, and in experiments II and III each plant was inoculated with five and 10 viruliferous aphids respectively, which were allowed to feed on the plants for 4 days. A further experiment was conducted combining experiments I, II and III. This enabled a more rigorous, statistical analysis using aphid numbers and infestation times as variables within a single experiment. Positive TBIA and ELISA results (more than twice

background readings) were found in all plants with symptoms and some symptomless plants, indicating the successful transmission of the virus. The glasshouse experiments showed that heavy yield losses could occur with no or only light symptom expression. There was a significant effect of BYDV-PAV infection for most of the varieties on growth and yield traits ($P < 0.05$).

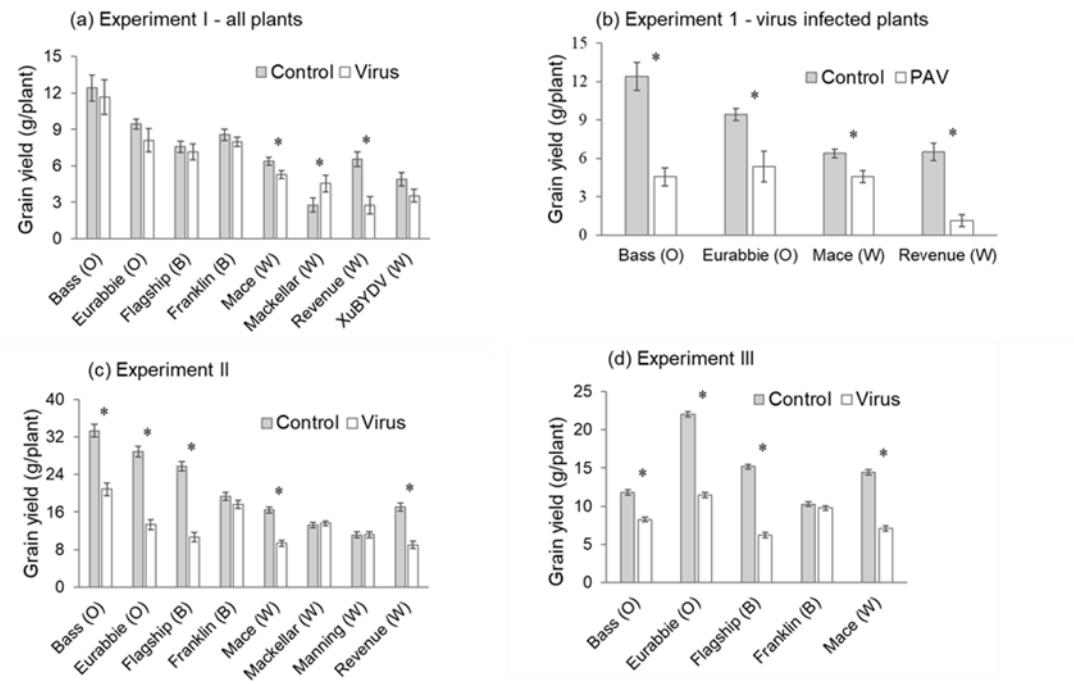



**Fig. 3.7** Average grain yield (g) in the BYDV-PAV treatment and control plants of wheat (W), barley (B) and oat (O) varieties. (a, b) Three aphids + 2 days inoculation, (c) five aphids + 4 days inoculation, (d) 10 aphids + 4 days inoculation. Standard error bars are shown on each column. * indicates a significant difference in grain yield between the control and virus infected treatments for that variety.

Larger numbers of viruliferous aphids increased the severity of visual BYD effects in all susceptible varieties in experiments II and III, and also increased the proportion of plants infected by the virus across all the trials, with no effect from different temperatures. These results support observations by Burnett and Gill (1976) that higher aphid loads lead to more plants becoming infected, and symptoms being more severe. There were few significant virus effects in experiment I when all plants were analysed, with only the varieties with the highest infection rates, Mace and Revenue, consistently showing a reduction in growth. Thus, any screening programme should use high aphid numbers to ensure high proportions of infected plants and significantly reduced biomass production in

susceptible varieties, leading to improved ability to discriminate rapidly between susceptible and resistant lines.

Some studies have used serological test methods such as TBIA and ELISA for detecting the presence of virus in cereal plants (Thackray *et al.*, 2005), while others have relied on visual symptoms (Sward and Kollmorgen, 1986). The present study showed that the scoring of visual symptoms was effective in barley and oats but not in wheat, as infected susceptible wheat plants did not show any visual symptoms at 4 WAI. However, plant growth was significantly affected in all susceptible varieties, making it a suitable indicator for virus infection. However, most of these wheat plants displayed symptoms by head emergence, while the symptoms in the barley varieties decreased in severity over the same time. Similar positive and false negative results have been reported elsewhere, with Chapin *et al.* (2001) reporting that 96% of stems with symptoms tested positive for BYDV-PAV, while Osler *et al.* (1985) found no visual symptoms in BYD-PAV-infected maize. Other researchers have stated that chlorosis is an unreliable symptom for evaluation of BYDV tolerance (Cheour *et al.*, 1989).

These results suggest that ELISA/TBIA are important techniques for screening programmes as they allow discrimination between uninfected and infected plants, which may not have obvious symptoms, particularly in wheat where symptoms develop only in later stages of plant development.

Reduction in growth parameters, such as tillering, plant height, biomass and grain yield, is often observed in susceptible virus-infected plants (Jiménez-Martínez and Bosque-Pérez, 2009). All susceptible varieties in the experiments here showed significant reduction in plant height after BYDV-PAV inoculation. Tiller numbers per plant were significantly reduced in both susceptible wheat and oat varieties, contributing to biomass and yield reduction when infected by the virus. However, the susceptible barley variety Flagship did not have a significant reduction in tiller numbers in any trial, but still had reductions in other growth parameters. In barley, tillering can be increased by BYDV infection, but in this case, most tillers develop poorly and produce sterile heads (GRDC, 2014).

BYDV infection caused significant reductions in biomass production of susceptible cereal varieties, but to a greater extent when inoculated with a higher number of

aphids. Chéour *et al.* (1993) found that in wheat, plant biomass became significantly reduced after viruliferous aphid inoculation. Symptom scores did not relate well to ELISA values; however, these authors observed a highly significant correlation between plant aerial biomass and log (1+) number of aphids. Higher aphid numbers might be useful for the deduction of yield loss with infection, so in trails to evaluate BYDV tolerance genotypes, inoculation with a higher number of viruliferous aphids should be effective.

Reduced kernel weight, kernels per square metre, kernels per spike, spikes per square metre and test weight are reported to be the major contributors to yield loss due to BYDV infection (Liu *et al.*, 2014). In the present study, BYDV-PAV infection had a clear effect on TKW compared to controls. A decrease in TKW has previously been observed in BYDV-infected oat (McKirdy *et al.*, 2002), barley (Edwards *et al.*, 2001) and wheat (Baltenberger *et al.*, 1987). Hoffman and Kolb (1998) also found the yield components such as grain number severely affected by virus infection. Although leaf yellowing was not profound in susceptible varieties under some circumstances, yield losses in wheat ranged between 28% and 82% across the experiments. Banks *et al.* (1995a) studied the yield effects of a range of levels of BYDV infection on six susceptible winter wheat genotypes in the field using release of viruliferous aphids and control using insecticide and showed the loss of yield increased with level of infection in the plots. Similar results have been reported by McKirdy *et al.* (2002), who found that yield loss can be up to 85% due to BYDV infection.

The yield of a cereal genotype decreased as the number of viruliferous aphids used for inoculation increased (Burnett and Gill, 1976). However, Skaria *et al.* (1984) observed that a higher number of viruliferous aphids did not affect yield. The present results suggest that dosage affects disease incidence and severity, but not grain yield, regardless of different temperature conditions.

Yield loss, rather than disease symptoms, reflect more accurately the tolerance of a cultivar (Gaunt, 1981). In virus–host systems (Albar *et al.*, 1998), virus resistance (defined as low virus titre) is generally associated with a reduction in symptoms and yield loss. In the present study, the *Ryd2* gene-containing barley variety Franklin had a lower level of infection than the susceptible varieties. It also

developed BYD symptoms, but mostly had no significant reduction in yield and yield components. Franklin may therefore be considered to be partially resistant to this isolate. These experiments also showed that the oat cv. Bass is tolerant in comparison to the susceptible variety Eurabbie.

Varieties classified as resistant possess genes that restrict viral infection and replication (Cooper and Jones, 1983). They have the advantage of reducing or eliminating hazardous pools of viral inoculum and minimising virus spread. The *Bdv2* resistance gene has been sourced from *Thinopyrum intermedium* translocation TC14 (Wang and Zhang, 1996), and has only minor negative effects in various wheat backgrounds on yield, kernel weight and biomass in the absence of virus (Rosewarne *et al.*, 2015). In the results here, BYDV-resistant wheat varieties Mackellar and Manning, which both carry this resistance gene (Ayala-Navarrete *et al.*, 2007), demonstrated their resistance in all experiments. These two cultivars showed good performance in all growth and yield contributing parameters because they had very low infection rates. Because of the diversity of effects of the known resistance and tolerance genes, it is recommended that screening for new, unknown genes should use a combination of methods focussing on symptoms, diagnostic tests (TBIA/ELISA scores) and yield performance.

## 3.5 Conclusions

In conclusion, leaf symptom expression following early viral inoculation differed according to cereal species: in oat leaves there was continuous leaf discolouration over time, in wheat there was no leaf discolouration at early stages of plant growth, but visual symptoms developed at later stages, and in barley a remission of symptoms over developmental time was evident. In wheat, progress can be made using combinations of resistance and tolerance genes. Leaf symptoms alone are unreliable to use in screening for new resistance genes in wheat, while restriction of virus multiplication, reduced loss of plant biomass and grain yield are more effective. For effectively screening BYDV resistance or tolerance under glasshouse conditions, use of a higher number of viruliferous aphids (10 per plant) over 4 days at the 2-leaf stage is recommended in order to ensure consistent inoculation.

# Chapter 4. Agronomical, biochemical and histological response of resistant and susceptible wheat and barley under BYDV stress[3]


## Abstract

Barley yellow dwarf virus-PAV (BYDV-PAV) is one of the major viruses causing widespread and serious damage to cereal crops. To gain a better understanding of plant defence mechanisms of BYDV resistance genes (*Bdv2* and *Ryd2*) against BYDV-PAV infection, the differences in agronomical, biochemical and histological changes between susceptible and resistant wheat and barley cultivars were investigated. We found that root growth and total dry matter of susceptible cultivars showed greater reduction than that of resistant ones after infection. BYDV infected leaves in susceptible wheat and barley cultivars showed a significant reduction in photosynthetic pigments, an increase in the concentration of reducing sugar. The protein levels were also low in infected leaves. There was a significant increase in total phenolic contents in resistant cultivars, which might reflect a protective mechanism of plants against virus infection. In phloem tissue, sieve elements (SE) and companion cells (CC) were severely damaged in susceptible cultivars after infection. It is suggested that restriction of viral movement in the phloem tissue and increased production may play a role in the resistance and defensive mechanisms of both *Bdv2* and *Ryd2* against virus infection.


## 4.1 Introduction

Barley yellow dwarf virus-PAV (BYDV-PAV) is transmitted by aphids and has been recognized as one of the most serious viral pathogens of the *Luteovirus* genus that systemically infects cereal crops (Jiménez-Martínez *et al.*, 2004). Infection with BYDV-PAV causes significant economic losses throughout the world (Huth, 2000; Ramsell *et al.*, 2008). The use of resistant or tolerant varieties is an effective solution and economical method for controlling BYD disease (Ordon *et al.*, 2004).

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Virus tolerance describes the capability of a host genotype to survive or recover from the damaging effects of virus infection and yield, while resistance is the plant's ability to restrict or prevent the infection of virus (Cooper and Jones, 1983). Wheat lines containing *Bdv2* gene showed less yellowing and lower viral titer than susceptible wheat lines when infected by BYDV (Kausar *et al.*, 2015). Similarly, barley cultivars containing *Ryd2* also have lower virus titre after BYDV infection, which leads to less visual symptom and grain yield reduction (Beoni *et al.*, 2016). Visual scoring of symptoms for BYDV-PAV resistance is not always useful as BYDV-PAV can multiply and spread in the plant without showing any visual symptoms (Horn *et al*., 2013). Whole-plant metabolite profiles can be altered by virus infection (Shalitin and Wolf, 2000; Xu *et al.*, 2008). Through virus infection, many plant defence pathways can be activated or suppressed (Lewsey *et al.*, 2010; Whitham *et al.*, 2006).

BYDV is transmitted in a persistent, circulative and non-propagative manner (Conti *et al.*, 1990; Masterman *et al.*, 1994) and its transmission occur when an aphid feeds on infected phloem and phloem cells and then transfers the viruses in its saliva to healthy plants (Walling, 2008). Virus spread usually starts from cell-to-cell (short distance movement). In the later phase, the virus enters into the vascular tissue, where it is transported rapidly via phloem cells. This is referred to as long distance movement (Hipper *et al.*, 2013; Waigmann *et al.*, 2004). Plants infected by virus undergo strong metabolic and ultrastructural changes, even when no visible symptoms are apparent (Yan *et al.*, 2008). Disease development in host plants is likely to induce substantial biochemical changes such as in protein, phenolics, carbohydrates, and these metabolic changes may favour or inhibit disease development (Ayres *et al.*, 1996). In certain plant host-pathogen interactions, these alterations may play a major role in contributing to disease resistance. In crop breeding, the response of biochemical compounds in plants has been helpful to select fungal and insect resistant genotypes (Lattanzio *et al.*, 2006).

In chilli plants inoculated with pepper leaf curl virus (PepLCV), total phenolic content was increased in resistant cultivars and was decreased in susceptible cultivars (Rai *et al.*, 2010). A positive correlation was observed in cocoa between cocoa swollen shoot virus disease (CSSVD) resistance and total phenolic contents at 3 months after inoculation (Ofori *et al.*, 2015). Rapid synthesis of phenolics and

their polymerization in the cell wall has been suggested as a plant defence response against infection (Sattler and Funnell-Harris, 2013; Matern and Kneusel, 1988), while low levels of phenolics may be linked to disease susceptibility (Yao *et al.*, 1995). However, there is no report on the relationship between total phenolic contents measured after BYDV infection and BYDV resistance in cereal crops.

Sugar metabolism is a dynamic process with both metabolic fluxes and sugar concentrations fluctuating strongly throughout plant development and in response to environmental signals for example circadian changes and biotic stresses (Bläsing *et al.*, 2005; Borisjuk *et al.*, 2003). In melon plants, cucumber mosaic virus infection causes a significant increase in the sugar content within the phloem (Shalitin and Wolf, 2000). An increase in sugar concentration in tobacco leaves was caused by potato leafroll virus (PLRV) infection inhibiting phloem loading; the increased sugar led to the inhibition of photosynthesis (Herbers *et al.*, 1997). Reduced translocation of sugar and other nutrient molecules to the root system limits root growth and function and thus affects plant growth and grain yield (Riedell *et al.*, 2003). Biotic stress can also inhibit chlorophyll synthesis (Funayama-Noguchi and Terashima, 2006; Šutić and Sinclair, 1991), resulting in reduced photosynthesis. To date, many studies have been done with BYDV but little information has been reported regarding changes of biochemical compounds caused by BYDV infection in wheat and barley.

The aim of the study was to assess the response of different agronomical, biochemical and cell ultrastructural changes after systemic BYDV infection of susceptible and resistant wheat and barley plants, and to provide a better understanding of resistance mechanisms against BYDV-PAV infections.

## 4.2 Materials and methods

### 4.2.1 Plant materials

Two wheat (*Triticum aestivum*) cultivars (Manning and Revenue) and two barley (*Hordeum vulgare*) cultivars (Franklin and Flagship) were used in this study. Manning and Franklin are the cultivars with known BYDV resistance, containing *Bdv2* (Ayala-Navarrete *et al.*, 2007) and *Ryd2* gene (Raman and Read, 1999), respectively.

### 4.2.2 Aphid colonies

A colony of bird-cherry aphid, Rhopalosiphum padi, was collected from a Tasmanian barley field trial in 2014 and reared on barley (cv. TAM407227-a BYDV susceptible genotype) in a cage at 20 °C ± 2 °C, 65 ± 5% RH, with a photoperiod of L14:D10 using cool white fluorescent light under 450 µmol-$m^{-2}s^{-1}$ photosynthethically active radiation (PAR).

### 4.2.3 Virus isolates

One isolate of BYDV-PAV was obtained from the University of New England, New South Wales (NSW), Australia and maintained in barley cv. TAM407227 in small cages under the same conditions as the aphid colonies. The virus isolate was periodically (6-weekly) transferred to new plants using R. padi in clip cages.

### 4.2.4 Plant growth and virus inoculation

Ten seeds of each cultivar were sown in 2 l plastic pots, which filled with pre-fertilized potting mixture. After germination, seedlings were thinned to five uniform and healthy plants in each pot. The plants were grown in a glasshouse, between September and November 2016. The average temperature was 23 $^{o}$C in daytime and 15 $^{o}$C at night with a relative humidity of 65 to 80%. At two-leaf stage, each plant was inoculated with BYDV-PAV using ten viruliferous adult aphids (Rhopalosiphum padi) in a clip cage. An inoculation access period of 120 h was used to ensure virus infection of all plants. Aphids were then killed by spraying 1ml/L solution of the insecticide Karate (Syngenta Ltd.).

### 4.2.5 Leaf samples for biochemical analyses

The most recent fully expanded leaves of both controls and inoculated plants were harvested at different growth stages, i.e. 3 and 6 weeks after inoculation (WAI), for various analysis. All biochemical parameters were measured using spectrophotometer (Genesys 10S UV-Vis).

#### 4.2.5.1 Photosynthetic pigments

Photosynthetic pigments were measured using the method of (Moran and Porath, 1980). 0.2 g leaf tissue was ground into powder with liquid nitrogen, then homogenised with 1 ml 100% N, N-dimethylformamide (DMF). Homogenized

samples were centrifuged at 10,000 x g for 10 min to gather the supernatant. Then 1 ml DMF was added again and samples were centrifuged. The supernatant was removed and another 1 ml DMF was added. The absorbance was recorded at 663 and 645 nm in a spectrophotometer. Calibration was done by using a blank of 100% DMF. Chlorophyll a, b and total chlorophyll were calculated by following formulas:

$$\text{Chlorophyll a (mg g}^{-1}\text{ tissue)} = \frac{[12.7(OD663) - 2.69(OD645)] \times V}{1000} \times W$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ tissue)} = \frac{[22.9(OD645) - 4.68(OD663)] \times V}{1000} \times W$$

$$\text{Total Chlorophyll (mg g}^{-1}\text{ tissue)} = \frac{[8.02(OD663) + 20.20(OD645)] \times V}{1000} \times W$$

Where OD, Optical density at respective nm, V, Final volume of chlorophyll extract, W, Fresh weight of the tissue extracted

#### 4.2.5.2 Measurement of total protein content

Total protein was estimated by using Bradford method (Bradford, 1976) and absorbance was recorded at 595 nm. Bovine serum albumin was used as standard. Protein contents in leaf samples were recorded as µg of protein per gram of leaf tissue.

#### 4.2.5.3 Phenolic content

Phenol content was measured using the method of Singleton *et al.* (1999). Fresh leaves (250 mg) were homogenized with 85% methanol. The extract was centrifuged at 3000 g for 15 min at 10 ºC and the supernatant was separated. Folin and Ciocalteu's reagent (2 ml) was added to each 2 ml of the supernatant. A sodium carbonate solution (7.5%, 2 ml) was added to each test tube and after 30–45 min, the absorbance was read at wavelength 725 nm against a reagent blank. A standard curve using gallic acid was generated to determine the concentration of total phenols in the unknown sample.

#### 4.2.5.4 Reducing sugar content

Reducing sugars were determined based on the method of phenol-sulphuric acid (DuBois *et al.,* 1956). 0.2 g fresh leaf was homogenized with deionized water and the extract was filtered. 2 ml of the solution were mixed with 0.4 ml of 5% phenol. Subsequently, 2 ml of 98% sulphuric acid were added rapidly to the mixture. The

test tubes were allowed to keep for 10 min at room temperature and placed in a water bath at 30 ºC for 20 min for colour development. Light absorption at 540 nm was then recorded with the spectrophotometer. Blank solution (distilled water) was prepared in the same way as above (Ammar *et al.*, 2009). Contents of reducing sugar was expressed as mg $g^{-1}$ fresh weight (FW).

### 4.2.6 Biomass production

Four plants (above ground) were randomly sampled from each treatment and replication at 6 WAI. After taking the fresh weight plant samples were kept in oven at 65 °C for 72 h before recording the weight of dry matter.

### 4.2.7 Enzyme-linked immunosorbent assay (ELISA)

Leaves from four plants of each treatment and replication were collected at 6 WAI for ELISA test. BYDV-PAV polyclonal antibodies (Sediag, France) were used in DAS-ELISA (Clark and Adams, 1977) to detect the virus in leaf tissues. Samples were prepared by grinding 1 g leaf tissue in phosphate buffered saline, pH 7.4, with 2% polyvinylpyrrolidone and 0.2% egg albumin in a ratio of 1:20. We used 2 healthy controls and 2 positive controls. All samples (control and BYDV-PAV inoculated leaves) and positive and negative controls were tested in duplicate. Microplates were read using a photometer (MR 5000 Dynatech) at wavelength 405 nm. Our ELISA cut-off value is 2 times of the negative control (healthy control) in each test. Samples with absorbance values greater than twice the mean of negative controls were considered positive (Clark and Adams, 1977).

### 4.2.8 Histological examination

Anatomical structure of infected and control wheat and barley leaves was examined with a light microscope using Leica DM500 (USA). Three biological replications were performed for each treatment. For microscopic examination, wheat leaves (2×2 $mm^2$) from both susceptible and resistant leaves were cassetted (Techno Plas, South Australia) using biopsy pads (Trajan Scientific and Medical, Victoria, Australia). Samples were then fixed in 10% neutral buffered formaldehyde (Confix, ACFC, Australian Biostain, Traralgon, Victoria, Australia) for 24 h and processed overnight using a standard 15 h overnight procedure in an ASP300S tissue processor (Leica Microsystems, Wetzlar, Germany). Samples were orientated on

the EG1160 (Leica), embedded in paraffin wax (Surgipath Paraplast, 39601006, Leica) and sectioned at 3 microns using Leica RM2245 microtome and adhered to microscope slides (Menzel Glaser, Braunschweig, Germany) for 20 min at 60 ºC. Sections were deparaffinised, rehydrated and stained using Jung autostainer XL (Leica) for haematoxylin (Harris' Haematoxylin, AHHNA, Australian Biostain) and eosin, dehydrated cleared and cover-slipped (Leica CV5030) using CV Mount (Leica, 046430011).

### 4.2.9 Determination of root growth

For root length measurement, five seedlings were grown in a 2 l plastic pot filled with pre-fertilized potting mixture. The plants were grown in a glasshouse with the average temperature of 15 ºC in daytime and 8 ºC at night. Plant was inoculated with 10 viruliferous aphids for 120 h. The experiment was terminated at 3 WAI and root length was measured. The roots were carefully washed with tap water to separate substrates. The longest root length (cm plant$^{-1}$) was measured as the distance from the base of the plant to the end of the longest root. Five biological replications were performed for each treatment.

### 4.2.10 Data analysis

The experiments used a randomized complete block design (RCBD) with three replications for each cultivar and five plants in each replicate. Data were analysed using software SPSS 20.0. Two treatment means (the values of virus infected and control plants) were subjected to paired t-test. The value was considered to be statistically significant when $P< 0.05$. All results were presented with mean ± SE from the replicates. Graphs were drawn using the Microsoft Excel program. We used ANOVA to test the effect of BYDV stress, cultivars and their interaction on biochemical parameters.

## 4.3 Results

### 4.3.1 Symptoms after inoculation

Typical symptoms appeared on virus infected plants included leaf discoloration and dwarfism. Leaf discoloration in both inoculated barley cultivars was visible within 3 WAI (Fig. 4.1). At 6 WAI we did not observe leaf discoloration in either of the

wheat cultivars, although the susceptible cultivar showed evidence of dwarfism at that time (Fig. 4.2).

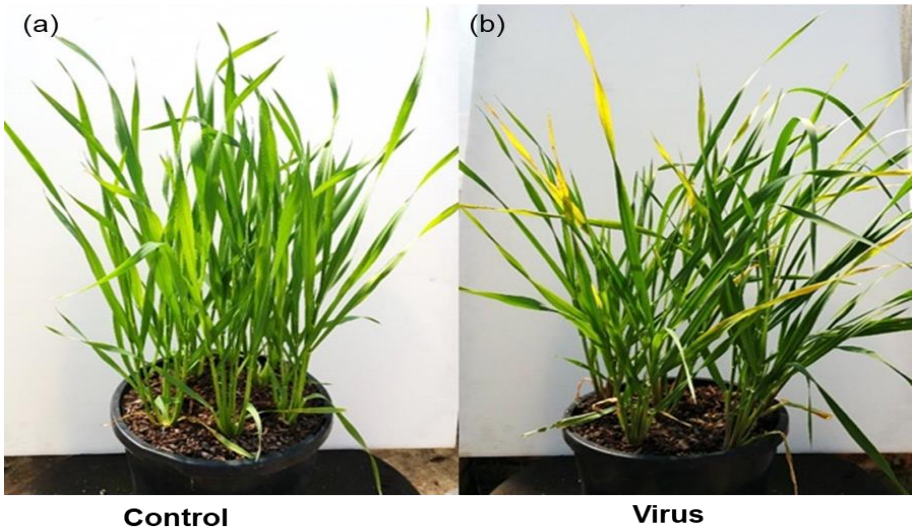



**Fig. 4.1** BYDV-PAV inoculated and control plants of susceptible barley cultivar Flagship at 3 WAI. (a) Control. (b) Virus infected.

### 4.3.2 Validation of inoculation

ELISA was used to confirm the virus infection of a plant, when ELISA values above the detection threshold (A 405>0.32) were assumed to illustrate the presence of virus particles. The virus extinction value was the highest in BYDV inoculated susceptible barley plant (Flagship) followed by susceptible wheat (Revenue) and the lowest value was detected in resistant wheat variety (Manning) (Fig. 4.3), suggesting that both the *Ryd2* gene of barley and the *Bdv2* gene of wheat reduced the viral load.

### 4.3.3 The effect of virus infection on root growth

At 3 WAI, all inoculated wheat and barley cultivars showed reduced root length compared to the control (Figs. 4.4a-d). Susceptible barley and wheat cultivars showed significantly greater reduction of root length by 41% and 36% for Revenue and Flagship, respectively. In contrast, resistant barley (Franklin) and wheat (Manning) only exhibited 7% ($p>0.05$) and 13% ($p<0.05$) reduction, respectively (Fig. 4.4e).

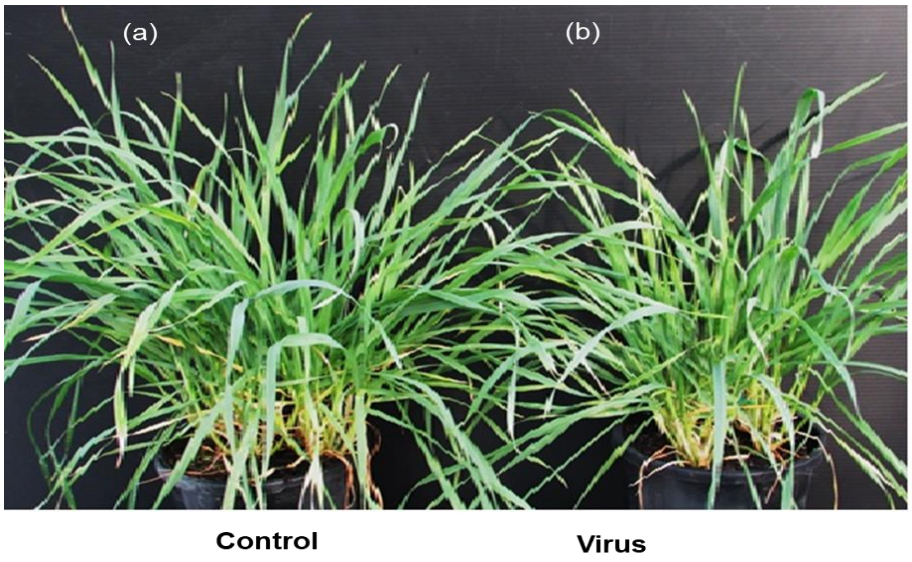



**Fig. 4.2** BYDV-PAV inoculated and control plants of susceptible wheat cultivar Revenue at 6 WAI. (a) Control. (b) Virus infected.

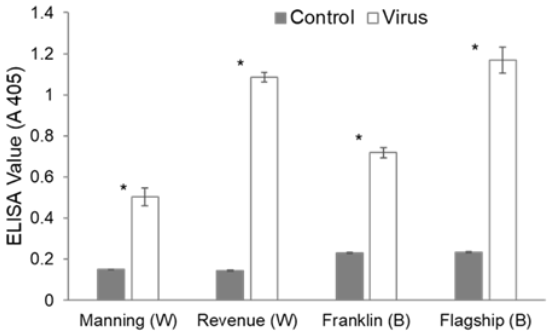



**Fig. 4.3** Mean virus extinction (A 405 nm) assessed in leaf extracts of BYDV-PAV inoculated and control plants of wheat (W) and barley (B) cultivars at 6 WAI. Data are mean ± SE, n = 9.

### 4.3.4 Photosynthetic pigments

At 3 WAI, the contents of photosynthetic pigments were significantly reduced in virus infected plants of both resistant and susceptible cultivars compared to the control. Greater reductions were found in susceptible ones. The average reductions in chlorophyll a, chlorophyll b and total chlorophyll were 33%, 50%, and 38%, respectively for Revenue, and 24%, 38% and 28%, respectively, for Flagship (Figs. 4.5a, b, c).

At 6 WAI, the difference in photosynthetic pigments between inoculated and control plants of the two resistant wheat and barley cultivars were insignificant. In

contrast, further reductions in photosynthetic pigments were found in the two susceptible cultivars (Figs. 4.5d, e, f and Table S1).

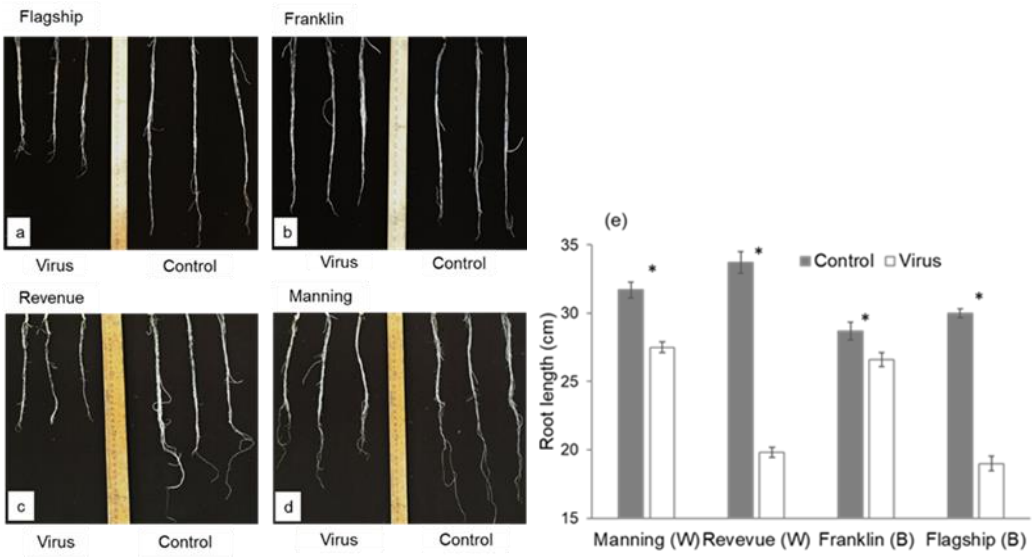



**Fig 4.4** Root appearance (a-d) and average root length (e) of BYDV-PAV inoculated and control plants of wheat (W) and barley (B) cultivars at 3 WAI. Data are mean ± SE, n=5.

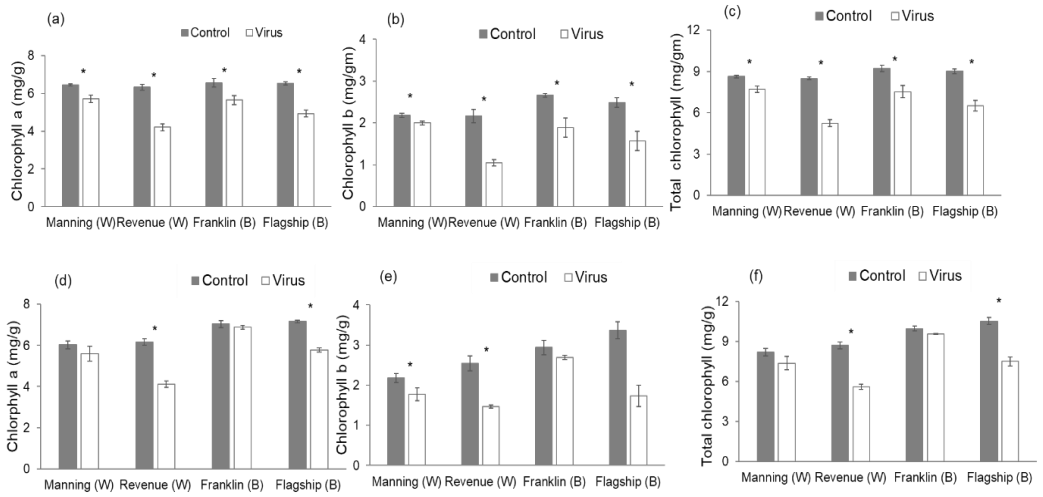



**Fig. 4.5** Average content (mg/g) of chlorophyll a, chlorophyll b, and total chlorophyll of BYDV-PAV inoculated and control plants of wheat (W) and barley (B) cultivars at 3 WAI (a-c) and 6 WAI (d-f). Data are mean ± SE, n=6.

### 4.3.5 Total protein

In wheat, virus infection caused a significant reduction in foliar protein contents only of susceptible wheat cultivar (Revenue) but not of the other cultivars at 3WAI ($P<0.05$) (Fig. 4.6a). At 6 WAI, both susceptible and resistant barley and wheat

cultivars showed significant reduction in protein content in virus infected plants. The reduction was observed more in susceptible cultivars Flagship (28%) and Revenue (27%) whereas the reductions in Manning and Franklin were only 9% and 11%, respectively (Fig. 4.6b).

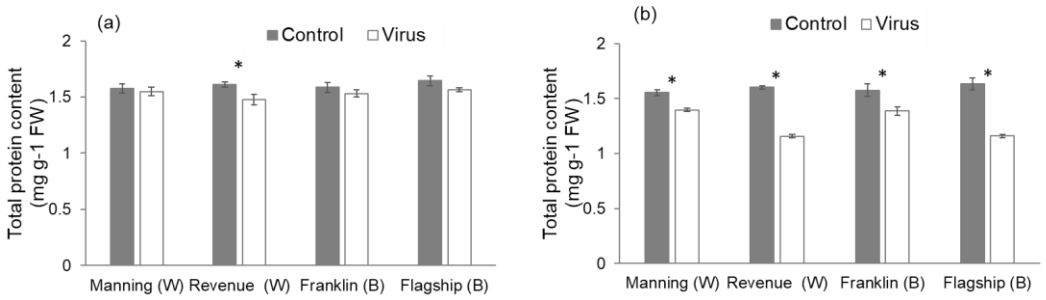



**Fig. 4.6** Average content (mg/g fresh weight) of total protein of BYDV-PAV inoculated and control plants of wheat (W) and barley (B) cultivars at 3 WAI (a) and 6 WAI (b). Data are mean ± SE, n=6.

### 4.3.6 Total phenol

At early stage (3 WAI) of virus infection, significantly differences in total phenol contents were found among cultivars (Table S1). However, total phenol contents were not significantly different between virus infected and control plants of all cultivars (Fig. 4.7a and Table 4.1).

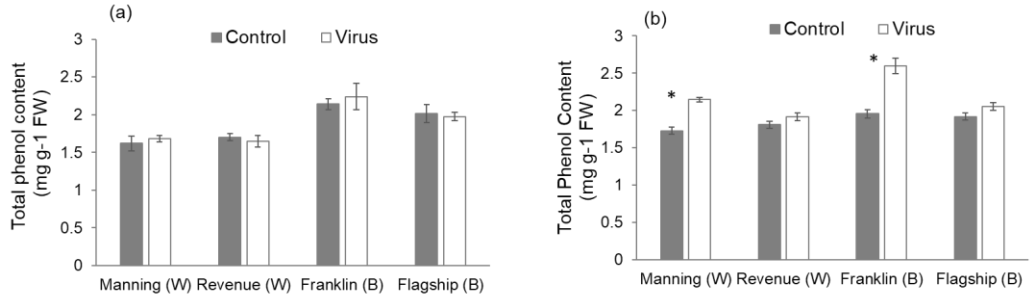



**Fig. 4.7** Average content (mg/g fresh weight) of total phenol of BYDV-PAV inoculated and control plants of wheat (W) and barley (B) cultivars at 3 WAI (a) and 6 WAI (b). Data are mean ± SE (n=6).

However, at 6 WAI, significant increases in total phenol contents were found in virus infected plants of all cultivars. The increase in total phenol contents was more

pronounced in the resistant cultivars (19-25%) than susceptible ones (5-6%) (Fig. 4.7b), Indicating a significant cultivar × treatment interaction (Table 4.1).

### 4.3.7 Reducing sugar

Figures 4.8a, b show that virus infection caused an increase in leaf sugar content of both susceptible and resistant cultivars. Wheat cultivars had lower sugar contents than barley cultivars earlier at 3 WAI, but they were similar to the barley cultivars at later stage (6 WAI). Significant cultivar × treatment interaction was found on sugar content at 6 WAI (Table S1). Susceptible cultivars showed a greater increase in reducing sugar contents in virus infected plants than resistant ones, being 9%, 26%, 13% and 35% at 6 WAI for Manning, Revenue, Franklin and Flagship, respectively (Fig. 4.8b).

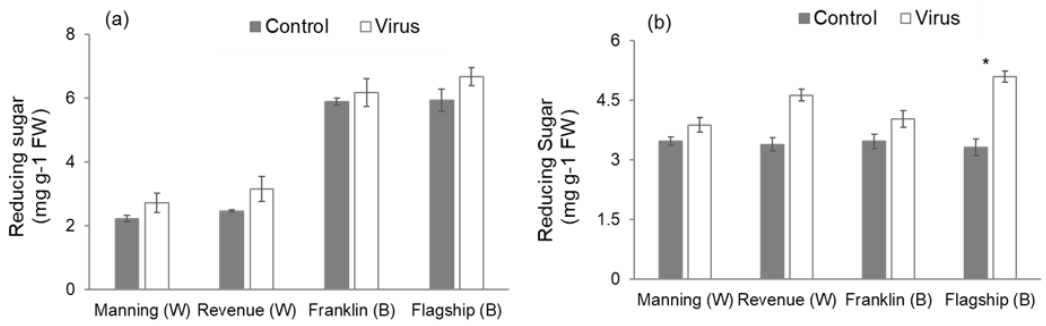



**Fig. 4.8** Average content of (mg/g fresh weight) reducing sugar of BYDV-PAV inoculated and control plants of wheat (W) and barley (B) cultivars at 3 WAI (a) and 6 WAI (b). Data are mean ± SE, n=6.

### 4.3.8 Biomass production

Significant differences in biomass were found in BYDV inoculated and non-inoculated control plants for all cultivars. The reduction of fresh weight varied with cultivar, with the lowest reduction occurring in the resistant wheat cultivar Manning (4%) and the highest in the susceptible wheat cultivar Revenue (41%) (Fig. 4.9a). Similar trend was found for the dry matter production. Greater reductions in dry matter were observed in Flagship (25%) and Revenue (22%) whereas the reductions in the resistant cultivars Manning and Franklin were only 6% and 9%, respectively (Fig. 4.9b).

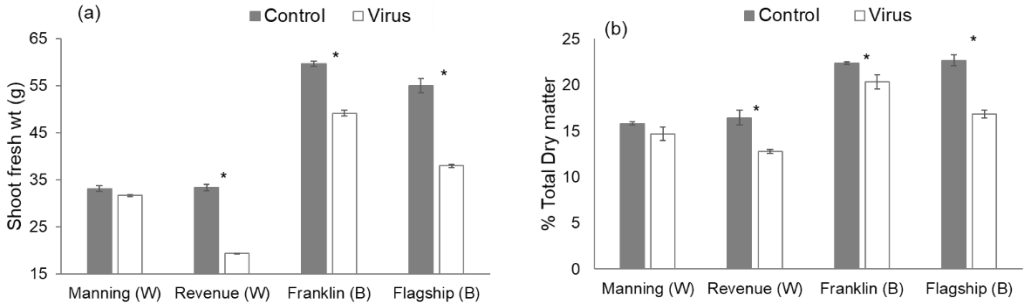



**Fig. 4.9** Average shoot fresh weight (g) (a) and relative dry matter (b) of BYDV-PAV inoculated and control plants of wheat (W) and barley (B) cultivars at 6 WAI. Data are mean ± SE, n=9.

**Table 4.1** Analysis of variance (F-value) for different biochemical parameters of wheat and barley cultivars as affected by main effects (cultivars and treatment) and their interaction between main effects

| Source of variance | At 3 weeks after inoculation | | | At 6 weeks after inoculation | | |
|---|---|---|---|---|---|---|
| | Chl a | Chl b | Total Chl | Chl a | Chl b | Total Chl |
| Cultivar | 4.9* | 3.14 | 4.01* | 37.54** | 11.92** | 34.39** |
| Treatment | 58.34** | 25.97** | 49.04** | 60.51** | 49.59** | 83.64** |
| Cultivar*Treatment | 3.34 | 1.04 | 1.75 | 11.40** | 7.13** | 12.51** |
| | **Protein** | **Phenol** | **sugar** | **Protein** | **Phenol** | **sugar** |
| Cultivar | 0.97 | 15.31** | 102.72** | 2.37 | 14.31** | 4.19* |
| Treatment | 8.16* | 0.06 | 7.60* | 86.46** | 46.18** | 70.58** |
| Cultivar*Treatment | 0.79 | 0.32 | 0.25 | 6.01** | 7.02** | 7.17** |

*P<0.05, **P<0.01

### 4.3.9 Alteration of leaf ultrastructure

Ultrastructural examinations of the phloem tissue of leaves from all cultivars were conducted at 6 WAI. The sections of leaf venial regions of non-inoculated plants showed a typical structure of vascular bundles in both wheat and barley. The sieve elements (SE) and the companion cell (CC) are well organised in the phloem tissue with each SE being adjoined by CC (Fig. 4.10a, c and 4.11a, c).

In virus infected barley plants, the phloem tissue of leaf veins consisted of smaller, denser and disorganised SE, with no adjacent CC. In addition, in the susceptible

barley cultivar Flagship the SE became necrotic, covered with dark stain and the CCs were degenerated (Fig. 4.10b).

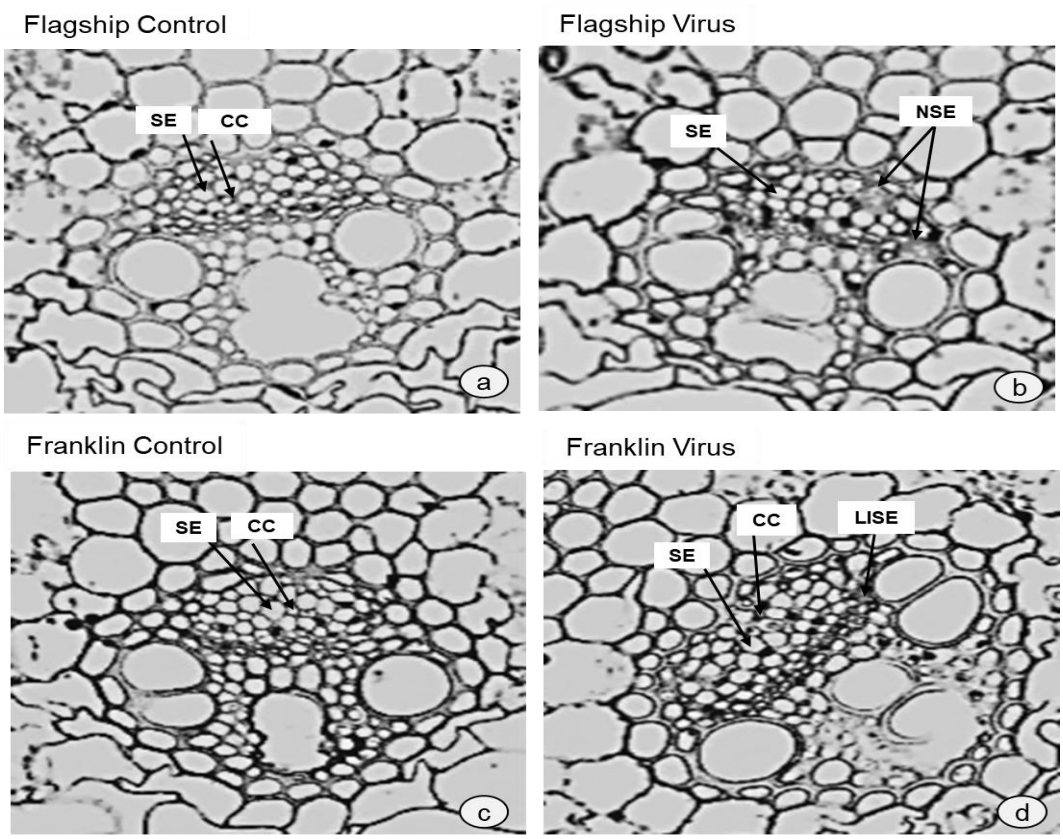



**Fig. 4.10** Transverse sections of foliar vascular bundles of susceptible (Flagship) and resistant (Franklin) barley cultivars. (a, c) from control plants and (b, d) from BYDV-PAV inoculated plants. Details are SE, sieve elements; CC, companion cell; NSE, necrotic sieve elements; LISE, little infected sieve elements. Data are mean ± SE, n=3.

Different results were observed in the resistant cultivar Franklin, which had normal SE with adjacent CC. Although these were looking almost the same as non-inoculated leaves, the CC seems to have reduced size compared with control. Necrotic regions were also observed in some vascular bundles in Flagship (Fig. 4.10b). In the susceptible wheat, BYDV-PAV inoculated leaf showed infected phloem parenchyma (IPP) and infected sieve elements (ISE) (Fig. 4.11b). The resistant wheat plant had similar cellular structures of SE and CC in virus infected and control leaves, thus vascular bundle was not affected in virus infected resistant wheat cv. Manning (Fig. 4.11d).

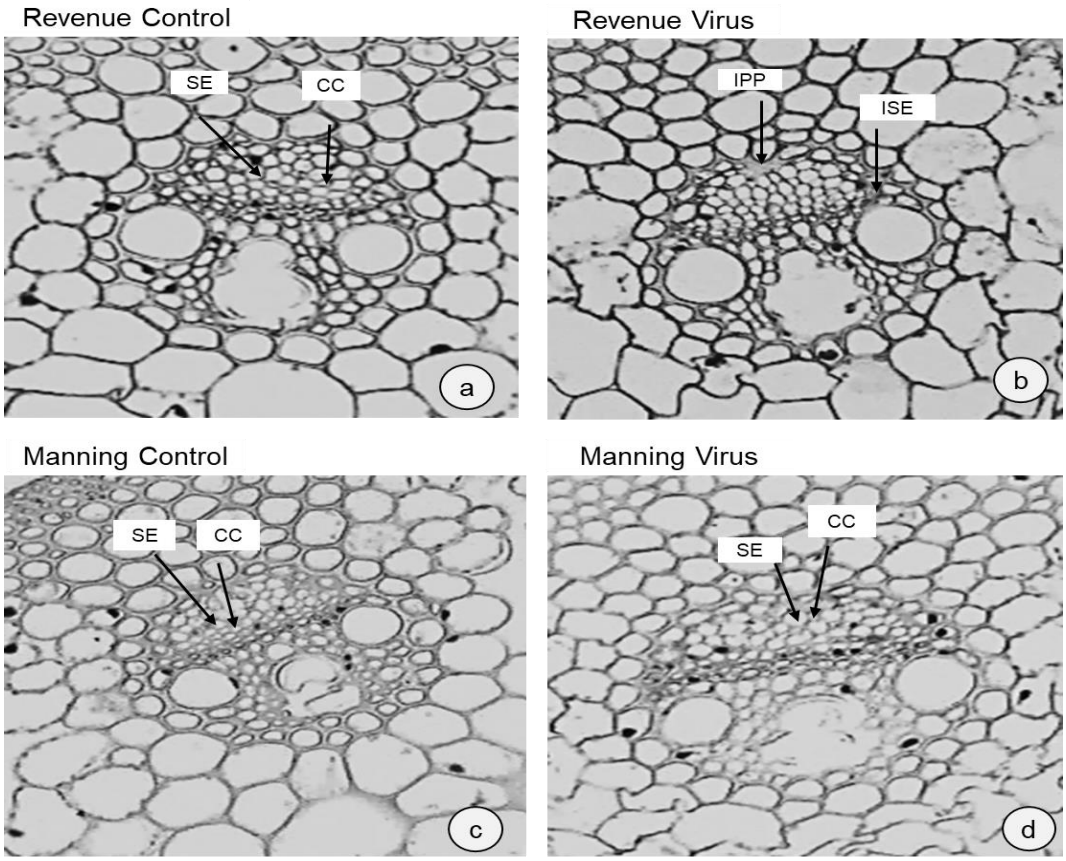



**Fig. 4.11** Transverse sections of foliar vascular bundles of susceptible (Revenue) and resistant (Manning) wheat cultivars. (a, c) from control plants and (b, d) from BYDV-PAV inoculated plants. Details are SE, sieve elements; CC, companion cell; ISE, infected sieve elements; IPP, infected phloem parenchyma. Data for mean ± SE, n=3.

## 4.4 Discussion

BYDV-PAV is one of the most destructive diseases of wheat and barley, which often causing significant yield losses when susceptible cultivars are grown (Jarošová *et al.*, 2013). To reduce BYD disease damage, the use of resistant cultivars is the most cost-effective and environmentally sound approach. For a better understanding of the mechanism of plant resistance to BYD disease, we investigated the changes of biochemical and ultrastructural characteristics in susceptible and resistant wheat and barley cultivars.

Reduction in chlorophyll content has been reported in many host plants infected with different viruses. The virus infection reduces chlorophyll contents of leaves producing chlorosis (Pineda *et al.*, 2008; Vimla and Shukla, 2009). In our experiment, at 6 WAI susceptible cultivars showed a significant decrease in the rate

of photosynthetic pigments. The reduced chlorophyll contents in susceptible cultivars are mainly due to a loss of leaf photosynthetic area and chloroplast disorders as observed in bean mosaic virus infected *vicia faba* leaves (Radwan *et al.*, 2008) while resistant gene can prevent the loss of chlorophyll in virus-infected leaves as shown in resistant tomato genotype after TMV infection (Fraser and Loughlin, 1980).

Yellow vein mosaic virus (YVMV) infection can cause enhanced activity of the chlorophyllase that attack chlorophyll and inhibit chloroplast development and chlorophyll synthesis in okra leaves (Ahmed *et al.*, 1986). The reduced photosynthesis capacity caused by reduced content of photosynthetic pigments contributes to the decrease in biomass production, which is shown in our studies as well as previous reports on BYDV infected cereals (Bukvayová *et al.*, 2006). Resistant cultivars infected with BYDV showed little effect on plant biomass, indicated resistant genotypes were able to maintain plant growth upon virus infection.

At early stages of plant development, screening of root traits can be used as a proxy for mature stages (Comas *et al.*, 2013). Greater root length contributed to enhanced grain yield of wheat by permitting more water extraction at grain filling stage (Manschadi *et al.*, 2006). Barley yellow dwarf virus (BYDV) affected the root elongation in wheat and barley cultivars differing in their response to BYDV, the reduction of total root length was less severe in the resistant cultivars than susceptible cultivars, which is shown in our results as well as previous reports on BYDV infected oat cultivars (Kolb *et al.*, 1991). Root length is associated with plant height (Steele *et al.*, 2006). Reduction in root length severely restricted water and nutrient absorption process, which may lead to the decreased of shoot growth (Riedell *et al.*, 2003).

Long-distance movement of virus particles is known to occur via the phloem, following the stream of sugar transport (Gilbertson and Lucas, 1996; Maule and Palukaitis, 1991). In sugarcane leaves infected by sugarcane mosaic virus (SCMV), sugar concentration is increased as a result of inhibited phloem transport (Addy *et al.*, 2017). Fiebig *et al.* (2004) measured sugar content in phloem sap of BYDV infected and non-infected wheat plants and observed that there was no significant

differences between control and infected plants. It is possibly BYDV blocked sugar movement into the phloem and consequently the rate of sugar movement was reduced, thus its concentration within the cells was higher. In this study, leaves infected with BYDV had significantly increased content of reducing sugar in susceptible cultivars. Likewise, Jensen (1969) and Jeson (1968) showed that BYDV infected plants had greater accumulation of carbohydrate in leaves, and a corresponding reduced chlorophyll content and rate of photosynthesis. The results of our study were similar to those found in a study of sunflower chlorotic mottle virus (SuCMoV) on sunflower in which infected leaves showed higher sugar accumulation and lower shoot biomass (Arias *et al.*, 2003). Misra and Jha (1971) observed an increase in reducing sugar in chilli leaves affected by mosaic virus, as did Gonçalves *et al.* (2005) in sugarcane leaves affected by sugarcane yellow leaf virus (ScYLV), possibly as a result of disruption of normal phloem transport or phloem loading. Shalitin and Wolf (2000) found that increased foliar sugar levels in melon plants following infection by cucumber mosaic virus were accompanied by increased respiration, which may lead to biomass reduction.

Protein components has been reported to be involved in plant pathogenic interactions (Carvalho *et al.*, 2006; Tornero *et al.*, 2002) with BYDV susceptible wheat cultivars showing significant reductions in protein content (Xu *et al.*, 2016). In the current experiment, significant reduction in protein content at 6 WAI was also found in resistant cultivars, which is different from the report of Sahhafi *et al.* (2012) that resistant wheat maintained higher protein content under wheat streak mosaic virus infection.

Phenolic compounds are often associated with plant responses to different stresses (Freeman and Beattie, 2008), with higher accumulation of phenols in resistant genotypes compared to susceptible ones in other virus/plant interactions (Siddique *et al.*, 2014; Singh *et al.*, 2010). Deposition of phenolics in plant cell walls might be a possible mechanism of virus resistance by playing key roles in increasing mechanical strength of host cell (Boudet*et al.*, 1995) and inducing cell wall lignification as lignin precursors (Lyon *et al.*, 1992). In the present study, at 3 WAI phenolic content was increased only in virus infected resistant genotypes. In addition, the total phenolic content was significantly increased in all infected resistant genotypes at 6 WAI but not in susceptible ones, suggesting that both

increased rate and quantity of phenolics might be components of the defence mechanism of *Bdv2* and *Ryd2* resistance genes.

In a vascular bundle, SE and CC participate in metabolic activities, and are responsible for long distance transport of minerals and assimilates. Viruses have been shown to affect both the structural and functional activities of the SE and CC (Lalonde *et al.*, 2001). BYDV is a systemic virus and its replication is almost entirely restricted within the plant phloem tissue (Irwin and Thresh,1990). BYDV particles are found exclusively in vasculature samples (Gill and Chong, 1975). Any restriction to phloem tissues will impact virus dispersal. However, there is also evidence of cell-to-cell movement of luteoviruses between nucleate cells of the phloem tissues (Mutterer *et al.*, 1999).

## 4.5 Conclusions

Although the damage to vasculature in BYDV infected plants remains to be quantified, we might speculate that accumulating viral load in the phloem leads to more widespread damage to the vasculature, and inhibition of sugar transport, which in turn inhibits root and biomass growth. The increased sugar content of leaves may also inhibit photosynthesis resulting in a further cycle of growth constraint. We hypothesise that the capacity to respond to virus with inhibitory phenolic compounds may be the basis of *Bdv2* and *Ryd2* resistance, limiting viral load and the cascade of pathological events described above.

# Chapter 5. Barley yellow dwarf virus infection affects physiology, morphology, grain yield and flour pasting properties of wheat[4]


## Abstract

Barley yellow dwarf virus (BYDV) is a phloem-limited virus that is persistently transmitted by aphids that causes significant yield losses in wheat. The current study was conducted to investigate the effects of BYDV in wheat on physiological and morphological traits, yield attributes and pasting properties of flour, and to determine any differences for these traits between susceptible and resistant genotypes under BYDV infection. A significant impact on physiological and morphological traits and yield was observed in plants inoculated at two-leaf stage, with a greater impact in the three susceptible genotypes compared to the resistant genotype. Yield reduction with two-leaf stage (GS12) inoculation was between 18-49%; yield reduction with mid tillering (GS25) inoculation was between 6-31%. There was a significant reduction in effective tiller number with both inoculation times, but thousand-kernel weight was only affected when inoculated early. Pasting properties were hardly affected by BYDV infection, and with genotype having a larger effect than infection. Tissue Blot Immunoassay (TBIA) and visual symptom score showed negative correlation, while grain yield showed positive correlation with all gas exchange parameters, chlorophyll fluorescence, leaf area and biomass weight. The results suggest that stomatal conductance, transpiration rate and chlorophyll fluorescence measurements are suitable for assessment of BYDV infection and screening BYD susceptible and resistant wheat genotypes.


## 5.1 Introduction

Wheat (*Triticum aestivum*) is one of the most important cereal crops in Australia and the most widely cultivated cereal worldwide (Arzani and Ashraf, 2017), with the global annual production being more than 700 million tonnes (WASDE 2018).

[4] This chapter has been submitted as: **Choudhury S,** Larkin P, Meinke H, Hasanuzzaman M, Johnson P and Zhou M Barley yellow dwarf virus infection affects physiology, morphology, grain yield and flour pasting properties of wheat. *Crop Pasture Sci.* Accepted

Global wheat production is impacted by virus infections (Velandia *et al.* 2010), barley yellow dwarf virus (BYDV) being one of the most harmful viruses. BYDV infection causes physiological disruption and serious economic losses (Trębicki *et al.*, 2015) There are several strains of BYDV, among them barley yellow dwarf virus- PAV (BYDV-PAV), which belongs to the genus Luteovirus of the family Luteoviridae, and is the most common serotype (Griesbach *et al.*, 1990). BYDV-PAV is persistently transmitted specifically by the aphids *Rhopalosiphum padi* and *Sitobion avenae* (Kaddachi *et al.*, 2014).

Yellowing or reddening of the leaf tips, particularly of the flag leaf is the most obvious symptom of virus infection (Kosova *et al.*, 2008). The severity of BYDV infection varies with crop species, genotypes and the age and physiological conditions of host plant at the time of infection (Loebenstein and Thottappilly, 2013). However, symptoms in BYDV infected wheat are not always obvious and may be confused with those caused by other biotic and abiotic stresses. Some BYDV infected plants show no symptoms (Irwin and Thresh, 1990), even though the presence of virus was established by serological test (Osler *et al.*, 1985). Wheat genotypes containing *Bdv2* gene showed less yellowing and lower viral titer compared to susceptible wheat genotypes, infected by BYDV (Kausar *et al.*, 2015). Plants infected by BYDV may show a significant reduction in plant biomass, leaf chlorophyll content, and grain yield (Jensen and D'Arcy 1995; McKirdy *et al.*, 2002). Banks *et al.* (1995a) studied the yield effects of a range of levels of BYDV infection on susceptible wheat genotypes in the field showed yield loss increased with the level of infection in plots. Similar results have been reported by McKirdy *et al.* (2000), who found that yield loss can be up to 80% due to BYDV infection. The yield losses caused by the BYDV infection can be up to 80%. Virus infection affects not only grain yield but also grain quality (Edwards *et al.*, 2001; Trębicki *et al.*, 2015). Among all the quality traits, pasting properties of starch are closely linked to the texture of cereal-based processed products (Zhang *et al.*, 2017). To the best of our knowledge, nobody has investigated the effect of BYDV infection on wheat flour pasting properties.

It is important to identify the most suitable physiological parameter to evaluate BYDV resistance. Plant grain yield will be the ultimate test but needs the entire life

cycle in the field to be assessed, which is time-consuming and a labour-intensive approach. Physiological traits such as Photosynthesis, transpiration rate, stomatal conductance, $CO_2$ assimilation, chlorophyll content, Fv/Fm and relative water content would be good measures. The variation of physiological processes due to viral diseases is one of the most important causes of decreased crop productivity across the world (Agrios, 1997). Yield reductions following virus infection might be due to decreased photosynthesis. The mechanisms through which viruses induce the reduction of photosynthesis and other physiological traits in host plants are not fully understood, nor are the mechanisms of BYDV resistance and tolerance. Photosynthesis might be impacted by reduction in chloroplast number and loss of chlorophyll content in various viral infections (Balachandran *et al.*, 1997; Ryšlavá *et al.*, 2003; Guo *et al.*, 2005a), with gas exchange parameters and chlorophyll fluorescence being possible, suitable indicators (Bonfig *et al.*, 2006; Berger *et al.*, 2007). Chlorophyll fluorescence and, mainly, the maximum quantum efficiency of light harvesting in PSII in dark adapted leaves, called Fv/Fm ratio, have proved to be a reliable indicator for abiotic and biotic stress tolerance (Duraes *et al.*, 2001). A significant decrease in Fv/Fm was observed in Nicotiana tabacum leaves infected by potato virus A and potato virus Y (Ryšlavá *et al.*, 2003; Zhou *et al.* 2004). However, this is not always the case as Eupatorium makinoi plants infected by a geminivirus showed no significant changes in the Fv/Fm ratio (Funayama *et al.*, 1997a).

Several methods are available for the control of BYDV. The aphid vector can be effectively controlled through insecticide treatments, but the timing is critical and multiple applications can make this approach expensive and environmentally damaging (Chain *et al.*, 2005). The use of plant varieties carrying genetic resistances is one of the most effective, economical, sustainable and frequently employed strategies to control viral infections (Nicaise, 2014). It is important to generate strategies for improving disease resistance in wheat. Uniform inoculation is essential for reliable selection of BYDV tolerant varieties, and requires the controlled application of reared viruliferous aphids. For effective breeding progress, natural infections in the field can be too unreliable. Controlled inoculation by infestation with viruliferous aphids also enables the assessment of particular virus isolates and impacts of inoculation at various developmental stages. It is important

to develop a reliable way to identify resistant/tolerant genotypes for large-scale selection in breeding programs. Despite the importance of BYDV, little attention has been given to physiological traits as potential selection criteria for resistance breeding.

The aims of this research are to investigate the effect of BYDV infection on physiological parameters, biomass, yield traits and dough pasting properties of wheat inoculated at different plant growth stages and their correlations in BYDV susceptible and resistant genotypes.

## 5.2 Material and methods

### 5.2.1 Plant materials and growing conditions

The field experiment was conducted at Tasmanian Institute of Agriculture, Launceston, Australia from September 2017 to January 2018. Four wheat genotypes (*Triticum aestivum* L. cv Mace, Preston, Wallup and BC Preston), were used in this experiment. BC Preston (Preston *4/Pontin13) is the only resistant genotype, being homozygous for the *Bdv2* resistant gene and is a BC3 derivative in the background of cv. Preston. The *Bdv2* gene is carried on the Pontin13 recombinant alien translocation, originally derived from *Thinopyrum intermedium* (Ayala-Navarrete *et al.*, 2013). Molecular marker (ByAgi) was used at every backcross and in the $F_3$ families to confirm homozygosity; and the effectiveness of the resistance in this and other backcross derivatives was confirmed in preliminary experiments (unpublished) and this study (Fig 1). All genotypes were grown in outside tanks (1.2 m x 1.0 m x 0.6 m) filled with a mixture of sandy loam soil and with plant spacing of 10 cm x 10 cm. The experiment was conducted with a randomized complete block design with three replications. Fifteen plants of each treatment were randomly selected for the measurements of all the physiological parameters, visual symptom score and virus detection by TBIA. All the genotypes were assessed on the same day. The plants that were assessed for TBIA were the same plants that were assessed for symptom severity and all the physiological and morphological parameters. Fertiliser was applied at sowing at the rate of 250kg/ha of 5:10:10:5 (N: P: K: S). An additional 75 kg N /ha was top-dressed in stem elongation stage (GS31). We used a water tray to supply water to the bottom of

each tank. In the bottom of each tank there was 50 mm of coarse gravel overlaid with drainage matting and the soil placed on top to a depth of 400 mm. The water level of each container was maintained at 75mm depth by fitting a float valve to the water tray. Excess water, from rainfall, flowed back to the water tray and out an overflow. Any water lost from the plant containers through evapotranspiration caused a local drop in the 750mm water level which was resupplied by the water tray. The lowest soil level remained fully saturated while the surface was dry. There was a gradient of moisture by depth between the top and bottom.

### 5.2.2 Aphid colony and virus Inoculations

Viruliferous aphid (*Rhopalosiphum padi*) colony were multiplied for six weeks in a growth chamber at 20 ± 2 °C under a photoperiod of 16L/8D on a sensitive wheat genotype "Revenue" infected with BYDV-PAV. Colony infection status was regularly tested using a Tissue-Blot Immunoassay (TBIA) (see below) to ensure that aphid colonies were viruliferous. There were three treatments: inoculation with BYDV-PAV at two-leaf stage (three weeks after sowing), inoculation with BYDV-PAV at mid tillering stage (five weeks after sowing); and control (protected from aphid infection). All the genotypes showed a similar phenology thus the inoculation was conducted at the same dates for all the genotypes. The inoculation was done by placing 5–10 aphids on the second leaf from the top of each plant of each treatment. To prevent the transmission of the aphids and to maintain similar growing condition in all plots, inoculated and control, were shielded with transparent aphid proof mesh. One week after inoculation, the mesh was removed and the plants were sprayed with the insecticide "Astound (alpha cypermethrin)" to kill all aphids.

### 5.2.3 Tiller sampling for virus detection

At flowering stage (GS 65), fifteen plants of each treatment were harvested to test for the presence of BYDV-PAV using TBIA. One tiller from each plant was assessed according to the procedure outlined in (Schwinghamer *et al.*, 2014). Each tiller was blotted onto nitrocellulose membranes, examined with polyclonal (BYDV-PAV) or monoclonal (BYDV-PAV) antisera (Agdia, Elkhart, Indiana USA), and visually evaluated under a dissecting microscope.

### 5.2.4 Assessment of BYDV infection

The severity of BYDV infection tested by TBIA was scored on a 0-4 scale. The scores took into account the number of vascular bundles infected by the virus. Score 0 represented no virus; score 1 represented very low level virus, number of vascular bundles (<10%) infected and very low intensity of staining of the vascular bundle; score 2 represented low number of vascular bundles (>10% to 25%) infected and low intensity staining of vascular bundle; score 3, represented moderate number of vascular bundles (>25% to 50%) infected and moderate intensity staining of vascular bundle; score 4 represented higher number of vascular bundles (>50%) infected and intensely stained vascular bundles.

### 5.2.5 Visual assessment of infection

Disease symptoms of BYDV infected plants were visually assessed at flowering stage. The severity of symptom development was scored on a 0-5 scale, which assessed the proportion of leaves showing red to yellow discoloration in the inoculated plants. A whole plant without symptoms scored '0', while a few (<20%) discoloured leaves scored '1'; Score 2 plants had approximately 20% of leaves affected; score 3 plants had 40%; score 4 had 60%; and score 5 had almost all (>60%) the leaves affected. Average visual symptom scores were calculated.

### 5.2.6 Measurements of photosynthetic gas exchange

At flowering stage of each genotype, the gas exchange was measured between 10:00 and 13:00 local time on a sunny and cloudless day for fifteen plants of each treatment. Net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular $CO_2$ concentration (Ci) and transpiration rate (E) were measured from the middle portion of fully expanded flag leaves using a Li-Cor 6400 portable photosynthesis system (Li-Cor, Lincoln, Nebraska, USA). Temperature was set at 20 °C with Tleaf reading. Photosynthetically active radiation (PAR) was set at 1500 µmol $m^{-2}s^{-1}$ inside the chamber, $CO_2$ was supplied artificially and was kept at concentration 400 µmol mol-1 inside the chamber with a stable flow rate of 500 µmol $m^{-2}s^{-1}$ (Singh *et al.*, 2014). Chamber fan speed was set to high as default. The chamber relative humidity was maintained in the range of 40-50% by adjusting the $H_2O$ scrub. The

sample and reference Infrared Gas Analyser were matched between every five measurements.

### 5.2.7 SPAD chlorophyll meter reading

Leaf chlorophyll content was measured for fifteen plants of each treatment using a SPAD-502 chlorophyll meter (Konica Minolta, Osaka, Japan). Measurements were recorded from the middle of the flag leaves at flowering stage (GS 65).

### 5.2.8 Chlorophyll fluorescence (Fv/Fm ratio)

The maximum quantum yield of PSII photochemistry (Fv/Fm ratio) of fifteen randomly selected plants of each treatment was measured at flowering stage (GS 65), using a modulated chlorophyll fluorometer OS1-FL (Opti-Sciences Corporation, Tyngsboro, MA, USA). Leaves were dark-adapted for 30 min prior to measurements. Measurements were done on the upper surface of the flag leaves.

### 5.2.9 Relative Water Content measurement

Relative water content (RWC) was determined according to Smart and Bingham (1974). For each replicate, five flag leaves were pooled, and their fresh weights (FW) were determined. The leaves were then immersed into water for twelve hours at room temperature to regain turgidity; the turgid tissue was then quickly blotted to remove excess water and then their turgid weights (TW) were measured. The samples were then dried in an oven at 56 °C for 24 h to determine the dry weights (DW). The RWC was calculated using the following formula:

RWC % = ((FW−DW)/(TW−DW))*100.

### 5.2.10 Measurement of leaf area

At flowering stage, flag leaves were randomly collected from 5 plants of each plot and leaf area was measured by using the Paton Electronic Planimeter (Paton Industries P/L, South Australia.). The area of the leaf was measured as the leaf is drawn through the scanning head. The scanning head was combined with a transparent belt conveyer with constant speed in order to measure the area of detached leaves.

### 5.2.11 Measurement of plant biomass and yield traits

Three plants were randomly sampled from each treatment and replication at flowering stage. Plants were dried for two days at 65 °C for 72 h before taking biomass. At maturity, ten plants were harvested from the centre of each replicate (30 plants in each treatment) for determining the number of effective tillers. Grain yield and thousand-kernel weight (TKW) were determined after threshing the mature spikes. Seeds were tested for pasting properties.

### 5.2.12 Measurement of pasting properties

The Rapid Visco-Analyser (RVA-4D, Newport Scientific, Australia) was used to measure the pasting properties. Pasting properties were measured using the method of Zhou and Mendham (2005). After harvesting and threshing, 10.0 g of cleaned grains were sampled from each genotype in each replication and ground on a Cylotech 1903 Mill. Afterwards, 4.0 g of the flour was dissolved into 25.0 g of 0.1M of silver nitrate (AgNO3) solution in an aluminium canister and mixed well before placing into RVA. To ensure the dispersion of the grist the RVA was used for 10 s at 960 rpm then reduced to 160 rpm for the test run. The initial temperature was 50 ºC, for 1.0 min, then elevated to 95 ºC for 3.7 min, held for 2.5 min and cooled to 50 ºC in 3.8 min and held for 2.0 min

### 5.2.13 Statistical analysis

Statistical analysis were performed with Statistical Analysis System (SAS) version 9.4 software using ANOVA followed by the Duncan's Multiple Range Test (DMRT) to evaluate the genotypes and treatments effect. The significance of correlations between different parameters was determined by bivariate correlations based on Pearson's correlation (two-tailed).

## 5.3 Results

### 5.3.1 Validation of Inoculation

BYDV infections were confirmed by TBIA, with susceptible genotypes showing higher levels of infection than the resistant one (Fig. 5.1a). When inoculated at two-leaf stage, the highest TBIA score was observed in susceptible wheat genotype Mace and Wallup (2.8) and Preston (2.6). Mace also showed the highest TBIA score

in plants inoculated at tillering stage (2.3) followed by Wallup (2.1) and Preston (1.8). BC Preston had the lowest infection scores at both inoculation stages, being 1.8 and 1.3 for two-leaf stage and mid tillering stage, respectively.

### 5.3.2 Development of Leaf Symptoms

Symptom expression was recorded at flowering stage. All genotypes except BC Preston developed strong leaf yellowing symptom indicating the lower field infection character of this genotype. Disease severity was greater when plants were infected at seedling stage (GS 12), causing the highest leaf discoloration of susceptible genotypes (Fig. 5.1b). *Bdv2* gene containing wheat genotype BC Preston showed a significantly lower disease symptom score (1.8) than Mace (3.8), Wallup (3.6) and Preston (3.2).

### 5.3.3 Photosynthetic gas exchange, chlorophyll content and Chlorophyll Fluorescence

Consistent with leaf symptom development data, BC Preston showed less reduction in photosynthesis than the three susceptible genotypes under BYDV stress. Mace showed relatively higher photosynthesis than the other three genotypes when grown under non-inoculated conditions (Fig. 5.2a). BC Preston showed a reduction in Pn only by 12% and 6% (compared with control) for early (GS12) and late (GS25) infection, respectively. Much stronger reduction was found in the three susceptible genotypes (approximately 44% in Wallup; 56% in Mace; 40% in Preston; under early infection).

Chlorophyll content (measured by SPAD meter) was significantly reduced in BYDV infected wheat across the genotypes, and more reduction was observed in plants inoculated at two-leaf stage than inoculated at mid tillering stage (Fig. 5.2b). The average reductions of leaf chlorophyll were 35 to 38% ($p<0.01$) for early and 24 to 33 % ($p<0.01$) for late inoculation whereas the resistant genotype BC Preston exhibited only about 16% and 10% ($p<0.05$) reduction when inoculated at early and late stages respectively.

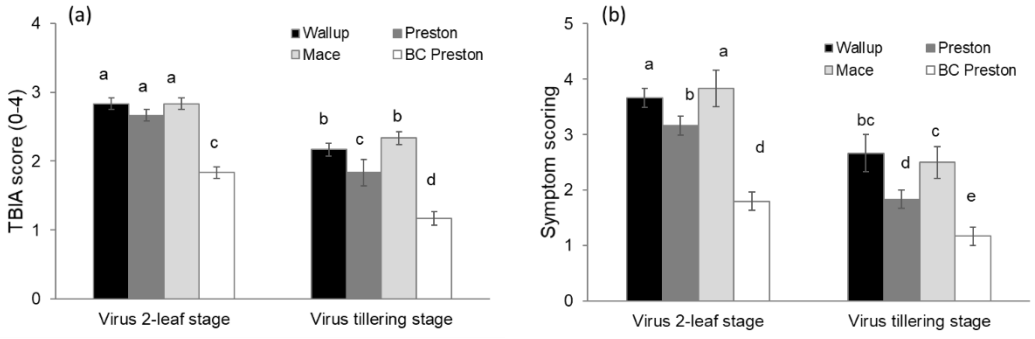



**Fig. 5.1** TBIA scoring (a) and BYD symptom severity scores (b) at flowering stage for four different wheat genotypes inoculated with BYDV-PAV at two different plant growth stages. Mean ± SE (n=15). Data labels by different letter is significantly different within and between treatments at P<0.05 by DMRT.

Similar to Pn, BYDV infection significantly reduced stomatal conductance (Gs) and transpiration rate (E) across the four genotypes (Fig. 5.2c, d). In BC Preston reduction in Gs was only by 10 to 20 % (from control), infected at tillering stage and two-leaf stage, respectively (Fig. 5.2c). When inoculated at two-leaf stage there was approximately 70% reduction in Gs observed in both Wallup and Mace; whereas when inoculated at tillering stage the reduction was approximately 60%. There was not much difference in reduction in E in Wallup and Mace (45%) at early infection, while the reduction was approximately 32% and 7% in Preston and BC Preston, respectively (Fig. 5.2d). Among all the gas exchange parameters, intercellular $CO_2$ concentration (Ci) was least affected with only 4 to 12% decrease when inoculated at two-leaf stage and 1-6% decrease, when inoculated at mid-tillering stage (Fig. 5.2e).

BYDV inoculation also reduced chlorophyll fluorescence (Fv/Fm ratio), with Fv/Fm values ranged from 0.81 to 0.82 among the genotypes under control conditions (Fig. 5.2f). However, a significant variation in the Fv/Fm values was found in BYDV infected plants (P<0.01; fig. 5.2f). The reduction in Fv/Fm value was approximately 20% in both Wallup and Mace; and approximately 14% in Preston, at early infection stage. Furthermore, lower reduction of Fv/Fm values was observed in BC Preston, which was as low as 2 and 5%, respectively, under late and early infection.

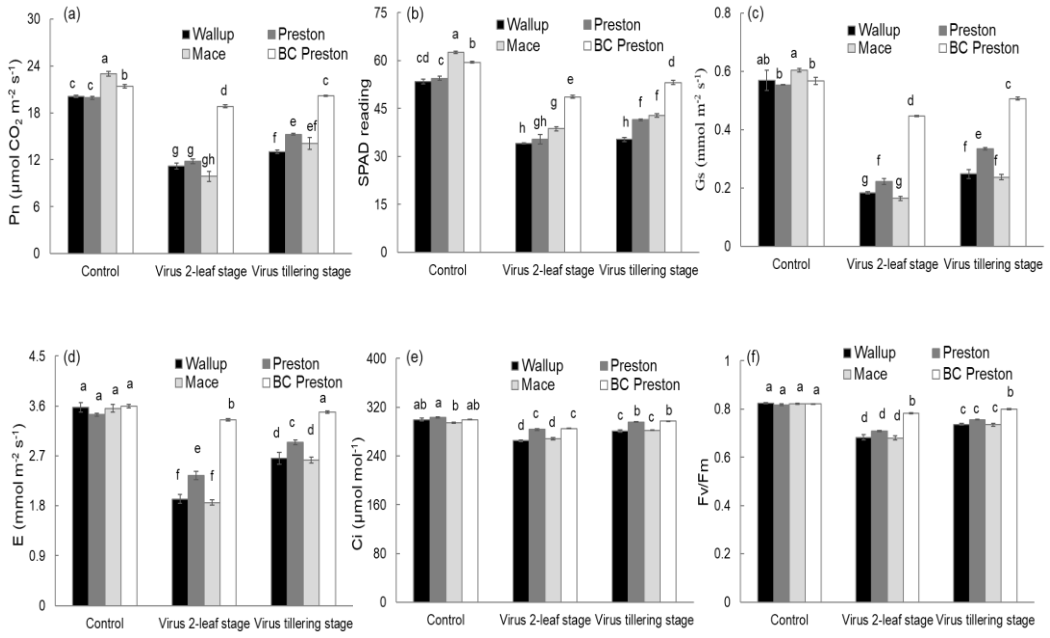



**Fig. 5.2** Net photosynthesis rate (a), leaf chlorophyll content (SPAD value b), Stomatal conductance (c), transpiration rate (d), Intercellular $CO_2$ concentration (e), chlorophyll fluorescence (f) of four different wheat genotypes under BYDV inoculated and control conditions. Mean ± SE (n=15). Data labels by different letters is significantly different at $P < 0.05$ by DMRT.

### 5.3.4 RWC, leaf Area and plant Biomass

BYDV infection caused significant reductions in relative water content in flag leaves of all susceptible wheat genotypes, ranging from 7% for Preston to 12% for Wallup when inoculated at two-leaf stage and from 4% for Preston to 6% for Mace when inoculated at mid-tillering stage. No significant change was found in the resistant genotype at late stage inoculation (Fig. 5.3a).

Flag leaf area was significantly ($p<0.01$) reduced in all wheat genotypes when virus was inoculated at two-leaf stage (Fig. 5.3b). Wallup showed the greatest reduction (42%) while BC Preston showed the lowest reduction (15%). Late infection (mid-tillering stage) caused less reduction in flag leaf area, being 18-29% for sensitive genotypes and only 6% for BC Preston.

Early BYDV infection resulted in a significant reduction in plant biomass across the genotypes (Fig. 5.3c). BC Preston showed the least reduction in shoot biomass, whereas Preston, Wallup and Mace were much more sensitive to BYDV treatment.

Even at early infection, BC Preston was able to maintain its biomass weight at about 75% of control. For Preston, Wallup and Mace biomass weight were reduced to 40, 45 and 50% of that in control, respectively. The same trends were shown when inoculated at mid tillering stage (Fig. 5.3c).

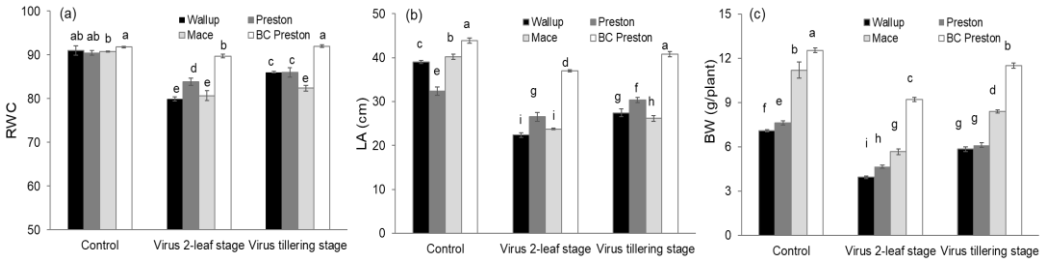



**Fig. 5.3** Relative water content (a), flag leaf area (b) and plant biomass weight (c) of four different wheat cultivars genotypes under BYDV inoculated and control conditions. Data labelled by different letters are significantly different at P<0.05 by DMRT.

### 5.3.5 Grain Yield, Effective Tiller Number and TKW

Grain yield was significantly reduced in BYDV infected wheat across the genotypes, especially when inoculated at two-leaf stage (Fig. 5.4a). The percentages of grain yield loss due to BYDV infection ranged from 14% in the resistant genotype BC Preston to 49% in the sensitive genotypes Mace and Wallup when inoculated at two-leaf stage. Late BYDV infection showed much less effect on grain yield with only 10% (Preston) to 31% (Wallup) reduction in sensitive genotypes and no significant reduction in the resistant one (Fig. 5.4a).

The number of effective tillers was significantly (P>0.01) reduced by BYDV infection in all wheat genotypes inoculated at two-leaf stage compared with control with much greater reduction in susceptible genotypes (Fig. 5.4b). Reduction in tiller numbers was highest in susceptible wheat genotype Mace (34%) followed by Wallup (21%) and Preston (19%) while the lowest was observed in BC Preston (5%). There was 4-12% (P>0.05) decreased in effective tillers per plant in susceptible wheat genotypes inoculated at tillering stage (Fig. 5.4b. However, the resistant wheat genotype (BC Preston) showed no significant change. The TKW was also significantly (P>0.05) affected by virus infection in all wheat genotypes (Fig. 5.4c), inoculated at two-leaf stage, ranging from 3 to 18%. BYDV inoculation at mid tillering stage showed no significant effects on TKW except for Preston.

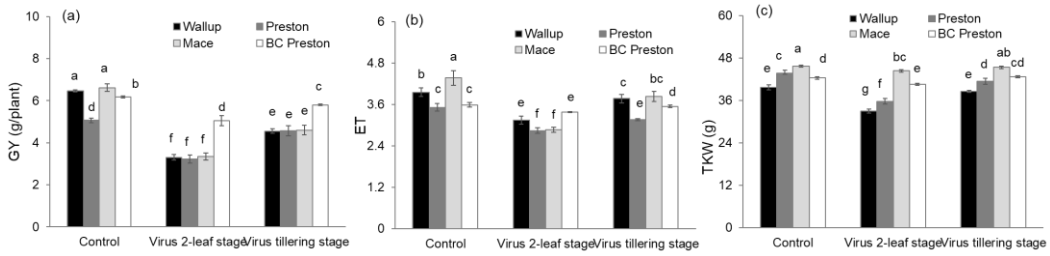



**Fig. 5.4** Grain yield (a), effective tillers (b) and thousand kernel weight (c) of four different wheat cultivars under BYDV inoculated and control conditions. Mean ± SE (n=30). Data labels by different letters is significantly different at $P < 0.05$ by DMRT.

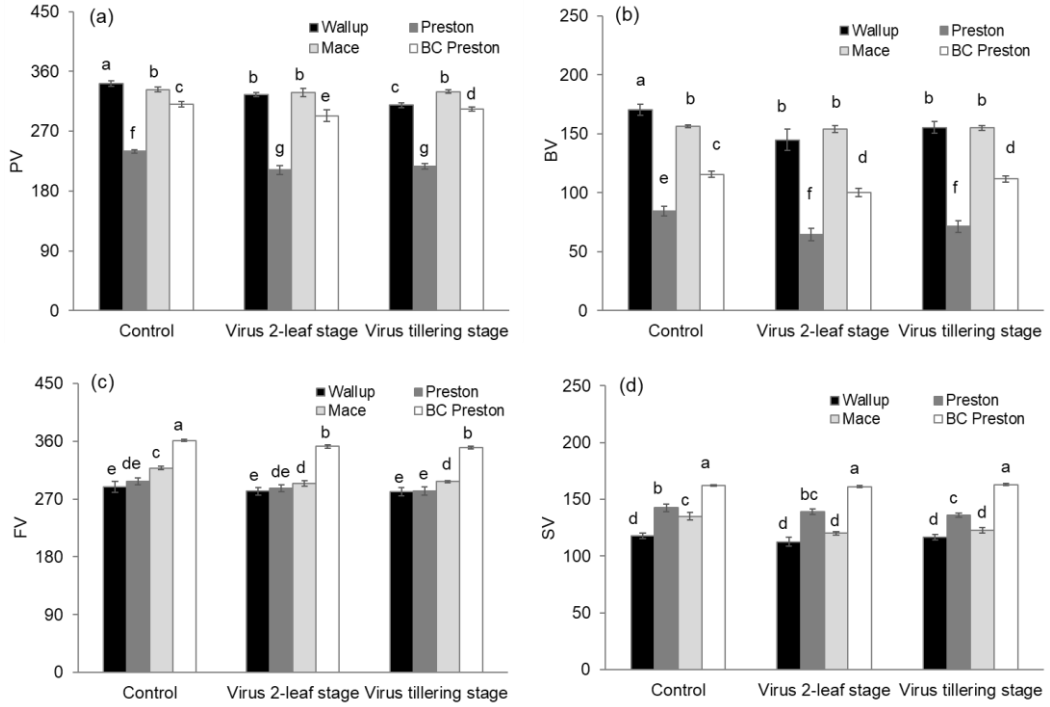



**Fig. 5.5** Peak viscosity (a), breakdown viscosity (b), final viscosity (c) and setback viscosity (d) of four different wheat cultivars under BYDV inoculated and control conditions. Mean ± SE (n=3). Data labels by different letters is significantly different at $P< 0.05$ by DMRT.

### 5.3.6 Effects of BYDV on pasting properties

Pasting parameters including peak viscosity (PV), breakdown viscosity (BV), final viscosity (FV) and setback viscosity (SV) of different wheat genotypes showed different responses under BYDV infection (Fig 5.5). PV (5%) and BV (15%) were significantly decreased in Wallup but had no effect on FV and SV compared to control. However, in Mace, FV (9%) and SV (11%) were significantly decreased

while showing no effect on PV and BV. Preston were significantly decreased PV (11%), BV (23%) and FV (4%) in BYDV inoculated plants compared to control. However, in BC Preston the reduction of different pasting properties ranged from 2-10%.

### 5.3.7 Correlation analysis

Pearson's correlation coefficients among BYDV treatments are listed in Table 5.1. Grain yield was positively correlated with all the physiological and morphological parameters and negatively correlated with TBIA and symptom score measured following two different inoculation stages. At early inoculation stage (GS-12) stomatal conductance and transpiration rate showed the highest correlation with grain yield, whereas at late inoculation stage chlorophyll fluorescence revealed the highest correlation with grain yield.

**Table 5.1** Correlation between relative grain yield and different physiological and growth traits of four wheat genotypes grown under field.

| Traits | Relative grain yield | |
|---|---|---|
| | Inoculated at GS12 | Inoculated at GS25 |
| Relative Pn | 0.87** | 0.76** |
| Relative E | 0.94** | 0.74** |
| Relative Gs | 0.94** | 0.84** |
| Relative Ci | 0.54 | 0.64* |
| Relative Fv/Fm | 0.93** | 0.88** |
| Relative SPAD | 0.89** | 0.87** |
| Relative LA | 0.73** | 0.60* |
| Relative RWC | 0.87** | 0.72** |
| Relative BW | 0.87** | 0.57* |
| TBIA | -0.83** | -0.35 |
| VSS | -0.88** | -0.62* |

Pn: net photosynthetic rate (μmol $CO_2$ $m^2s^{-1}$), E: transpiration rate (mmol $m^2s^{-1}$), Gs: stomatal conductance (mmol $m^2s^{-1}$), Ci: intercellular $CO_2$ (μmol $mol^{-1}$), Fv/Fm: chlorophyll fluorescence, SPAD: chlorophyll content, LA: leaf area, RWC: relative water content (%), BW: biomass weight. *: significant at 0.05 level; **: significant at 0.01 level.

## 5.4 Discussion

In this study, we investigated the effect of BYDV-infection on different physiological parameters and yield traits following inoculation at two different developmental stages. TBIA results indicated successful viral infection following both early and late inoculation. The study showed that the proportion of infected plants depended on plant genetic background. The experiment showed that earlier virus infection led to a greater impact on physiological parameters, and subsequently on plant biomass and yield. The experiment showed that heavy yield losses could occur in susceptible genotypes following both early and late infection. The resistant genotype was less affected by virus infection than susceptible ones, including its recurrent parental background.

The photosynthetic system is the physiological basis of crop growth and yield (Sun et al., 2009). Reduction in photosynthetic rate due to virus infection is associated with physical damage to the chloroplast structure and the deterioration of its membranes (Fraser and Fraser, 1987). BYDV infection caused significant reductions in Pn of susceptible wheat genotypes and showed higher negative correlation with symptom score likely because virus infection can reduce green leaf areas or cause transport blockage and accumulation of photosynthate in infected leaves. This abnormal accumulation may inhibit further photosynthesis by disrupting chloroplast structure, reducing $CO_2$ diffusion or light availability, and may be accentuated by phloem necrosis (Esau *et al.*, 1967). Jensen (1969, 1968) found that BYDV infected plants had greater accumulation of carbohydrate in leaves possibly because of disruption of normal phloem transport, and a corresponding reduced chlorophyll content and rate of photosynthesis. Pn reduction was observed in *Eupatorium makinoi* leaves infected by a *geminivirus* and it is suggested that the reduction may be due to the decline of chlorophyll per unit leaf area in infected leaves (Funayama *et al.*, 1997b). In our results, chlorophyll content of plants infected with BYDV was reduced significantly more in susceptible genotypes than the resistant genotype. Similar results were found in a study of *rice tungro virus* on rice plants, wherein virus infected resistant genotypes showed minimal loss of chlorophyll (Jabeen *et al.*, 2017). Reduction in chlorophyll contents

caused by virus infection might be a result of chlorophyll degradation (Liu *et al.*, 2014) or chlorophyll synthesis inhibition (Shimura *et al.*, 2011).

During photosynthesis, stomata play a critical role in $CO_2$ assimilation and C fixation that ultimately contribute to increased plant biomass and grain yield (Hetherington and Woodward, 2003). A decrease in stomatal conductance (Gs) and transpiration rate (E) was observed in grapevine leaves affected by *grapevine leafroll virus* (Bertamini *et al.*, 2004), in radish leaves infected by *turnip mosaic virus* (Guo *et al.*, 2005b), and in tobacco leaves affected by *potato virus Y* (Spoustová *et al.*, 2013). Stomata were less opened in sugarcane leaves inoculated with ScYLV (Lehrer and Komor, 2008). In this study, stomatal closure was evident as a drastic reduction in stomatal conductance, which presumably acted as a major factor in reducing net photosynthesis (Pn) in BYDV susceptible genotypes. BC Preston showed minimal decrease in Gs, as well as transpiration rate, even when inoculated early. Our results also showed a positive and significant correlation between E and Gs in all wheat genotypes tested.

Virus infection in susceptible plants induced reduction in photosynthetic traits including chlorophyll fluorescence (Fv/Fm ratio) and $CO_2$ assimilation (Ci) (Rys *et al.*, 2014). Chlorophyll fluorescence (Fv/Fm ratio) provides basic information regarding photosynthetic apparatus (Rapacz and Hura, 2004). In our result, light interception by PSII measured by the Fv/Fm ratio was significantly reduced in susceptible wheat genotypes, suggesting virus infection destroys functional photosynthetic reaction centers leading to chlorophyll degradation (lower SPAD values). In addition, BYDV infection also led to Ci reduction in susceptible wheat. The reduction of Pn in wheat leaves infected with *wheat streak mosaic virus* (WSMV) was associated with reduced Ci (Pradhan *et al.*, 2015).

BYDV infection severely reduced leaf areas of susceptible wheat plants compared to the control. *Cauliflower mosaic virus* (CaMV) caused significant reduction of the leaf areas of *Brassica rapa* and *Arabidopsis thaliana* plants (Doumayrou *et al.*, 2013). Banana plants infected with *Banana bunchy top virus* (BBTV) showed apparent significant decrease in leaf areas at 50 days after infection (Hooks *et al.*, 2008), leading to reductions in light interception (Kumar *et al.*, 2012). In our results, susceptible genotypes infected with BYDV had significantly reduced leaf area,

photosynthetic efficiency, and biomass weight. The resistant genotype infected with BYDV showed little effect on plant biomass.

It has been established that *Bdv2* confers resistance in the sense of reducing but not eliminating viral load (Banks *et al.*, 1995b), but in addition it seems to reduce the efficiency of transmission to plants (Ayala-Navarrete *et al.*, 2009; Jahier *et al.*, 2009). The reduced virus load in this study results in reduced effects on most of the physiological and morphological parameters measured.

In our results, two susceptible genotypes Wallup and Mace showed greater yield reduction (>30%) at both early and late infection. However, the reduction of yield was more pronounced with earlier inoculation as has been frequently observed with BYDV (Smith and Sward, 1982; Thackray *et al.*, 2009; Finlay and Luck, 2011; GRDC 2013). BYDV infection at later developmental stage has less time to disrupt plant physiological parameters, thus resulting in less yield reduction. Grain yield reduction in wheat plants infected with BYDV was mainly a consequence of reduced number of effective tillers per plant (El-Yamani and Hill, 1990) rather than TKW, especially when BYDV infection happened at a later stage. BYDV-resistant genotype BC Preston, which carries the resistance gene *Bdv2*, showed good performance in physiological and yield contributing parameters with low levels of infection rates.

BYDV infection had minimum effects on grain flour pasting properties. The difference between genotypes was greater than the effects of the infection, confirming that pasting properties are largely influenced by genotypes (Zhou *et al.*, 2008).

## 5.5 Conclusions

The present study suggests that gas exchange parameters (Pn, E and Gs), chlorophyll content, Fv/Fm, leaf area, RWC and plant biomass under BYDV stress could all be used as reference indicators for selecting BYDV resistant genotypes. However, Fv/Fm is relatively simple and rapid thus is likely to be more efficient in screening a large number of genotypes. Both TBIA and VSS showed significant correlation with grain yield. However, both cannot be scored at early growth stage and TBIA requires more time.

# Chapter 6. Genome wide association study reveals novel genes for barley yellow dwarf virus resistance in wheat[5]


## Abstract

Barley yellow dwarf (BYD) is an important virus disease that causes significant reductions in wheat yield. For effective control of *barley yellow dwarf virus* through breeding, the identification of genetic sources of resistance is key to success. In this study, 335 geographically diverse wheat accessions genotyped using an Illumina iSelect 90K SNP bead chip array were assessed for BYD resistance in different environments. A genome-wide association study (GWAS) performed using all the generalised and mixed linkage models (GLM and MLM, respectively) identified a total of 36 significant marker-trait associations, five of which were consistently detected in two models. These five novel QTL were identified on chromosomes 2A, 2B, 6A, 7A and 7B and associated with markers IWA3520, IWB24938, WB69770, IWB57703 and IWB65432, respectively. Several wheat genotypes (H-151, H-20, H-205, H-027, H-023 and H-056) showed consistently higher resistance in different field trials. None of them contained the previously described *Bdv2*, *Bdv3* or *Bdv4* genes for BYD resistance. These genotypes will be used in further research to confirm and characterise the QTL identified for BYD resistance.


## 6.1 Introduction

Barley yellow dwarf (BYD) is one of the most destructive wheat diseases worldwide and are caused by phloem limited *luteoviruses* recognised as *barley yellow dwarf viruses* (BYDV) (Miller *et al.*, 2002). The virus belongs to the Luteoviridae family and is transmitted by different aphid species. BYDV is divided into different distinct serotypes, based on the vector specificity and sequences of the virus. The most damaging serotype is BYDV-PAV (Lister and Ranieri, 1995),

[5] This chapter will be submitted as: **Choudhury S**, Larkin P, Xu R, Hayden M, Forrest K, Meinke H, Hu H, Zhou M and Fan Y Genome wide association study reveals novel genes for barley yellow dwarf virus resistance in wheat. Submitted to *BMC genomics*

which is transmitted by the aphids *Rhopalosiphum padi* and *Sitobion avenae* (Kaddachi *et al.*, 2014).

Symptoms of BYDV infection in wheat vary among cultivars and environments with the major ones being leaf discoloration, reduced plant growth and grain yield. Yield losses in wheat are estimated to be 27-45 kg/ha for each 1% increase in BYDV incidence (Banks *et al.*, 1995). YDV disease can be partially controlled through management practices such as time of sowing and the application of insecticides. However, breeding for resistant or tolerant cultivars is the most efficient and environmentally sound approach to prevent yield losses (Ordon *et al.*, 2004).

Cereal crops are most vulnerable to BYDV infection during early growth stages. Yellowing or reddening of leaf blades along the vascular bundles, especially at the leaf tips, and plant dwarfing, are the main symptoms of YDV disease in wheat (Kosova *et al.*, 2008; Choudhury *et al.*, 2018). These symptoms are positively correlated with the virus titre as measured by enzyme-linked immunosorbent assay (ELISA), which is an indicator for disease susceptibility (Horn *et al.*, 2013). In resistant plants, virus multiplication is reduced. The evaluation of wheat for BYD resistance using aphid inoculation and ELISA analyses is both laborious and costly. Marker assisted selection (MAS) of known resistance loci would allow quicker progress in breeding for wheat with BYD resistance.

Genetic mapping of bi-parental populations using molecular markers has been used to identify and characterise a number of QTL in common wheat for BYDV-PAV resistance. These include 22 QTL identified from the Opata × Synthetic recombinant inbred population (RIL) population and seven QTL from the Frontana × INIA66 RIL population (Ayala *et al.*, 2002) with one of the QTL from the Frontana × INIA66 population being located on 7DS at the same position of the *Bdv1* gene identified from a wheat cultivar Anza (Singh *et al.*, 1993). In addition, three BYD resistance genes from *Thinopyrum intermedium* (intermediate wheatgrass), called *Bdv2*, *Bdv3* and *Bdv4*, have been introgressed into common wheat background via chromosomal translocations (Zhang *et al.*, 2009). *Bdv2* was first introduced as a 7D-7Ai#1 translocation (Banks *et al.*, 1995), *Bdv3* as a 7B-7Ai#1 translocation (Crasta *et al.*, 2000) and *Bdv4* as a 2D-2Ai-2 translocation

(Zhang *et al.*, 2009). Evidently the different BYD resistance genes in Th. intermedium have different isolate specificities (Francki *et al.*, 2001) and possibly diverse mechanisms of action such as interfering with virus multiplication (Sharma *et al.*, 1989) or reducing cell-to-cell movement (Anderson *et al.*, 1998).

While effective sources of resistance to BYD have been identified in wheatgrasses, few have been reported in common wheat (Ayala-Navarrete and Larkin, 2011). The only reported gene for BYD resistance in wheat, *Bdv1*, and associated with the rust resistance *Lr34/Yr18* gene complex, may reduce leaf symptoms (Singh *et al.*, 1993) but fails to confer any protection for biomass or grain yield (Ayala *et al.*, 2002; Ayala-Navarrete and Larkin, 2011). In this study we performed a genome-wide association study on a geographically diverse collection of 335 bread wheat accessions to identify new sources of resistance to BYD.

## 6.2 Materials and Methods

### 6.2.1 Aphid colony

Bird-cherry aphid, *Rhopalosiphum padi*, was collected from a Tasmanian barley field trial in 2014 and raised on oat (cv. Eurabbie-a BYDV susceptible genotype) in small cages at 20 °C ± 2 °C, 65 ± 5% RH, with a photoperiod of L14:D10 by cool white fluorescent light under 450µmol.$m^{-2}s^{-1}$ photosynthethically active radiation.

### 6.2.2 Collection and maintenance of virus isolate

The isolate of BYDV-PAV was obtained from the University of New England, New South Wales (NSW), Australia and maintained in oat cv Eurabbie in small cages under the similar conditions as per the aphid colonies. The virus isolates were periodically (8-weekly) moved to new plants with *R. padi*. To ensure viruliferous aphid colonies, the infection status was frequently tested using ELISA which uses polyclonal BYDV-PAV antibodies (Clark and Adams 1977). Results were assessed by using a Multiskan RC plate reader with GENESIS software (Lab Systems). All samples were tested twice. Samples with absorbance values greater than twice the mean of negative controls in one or both samples were considered positive.

### 6.2.3 Plant materials, virus inoculation and phenotyping

A total 335 wheat accessions obtained from China and the Australian Grains Genebank were used in this study. These accessions were evaluated for BYD resistance in 2016 and 2017 at the Tasmanian Institute of Agriculture, Launceston, Australia. Each accession was grown in triplicate in hill plots in a randomised complete block design. Five seeds were sown in each hill plot. Each hill plot was inoculated at the 2-leaf stage (Zadok's Growth Stage 12) with BYDV-PAV using ten to fifteen viruliferous adult aphids (*R. padi*) (Choudhury *et al.*, 2018). An inoculation access period of 120 h was used to ensure virus infection of all plants before aphids were killed by spraying 1ml/L solution of the insecticide Karate (Syngenta Ltd.). The accessions were also evaluated in the field in 2017 under natural BYDV infection at the Burlington Road at Cressy Research Station, Tasmania (-41.709400 N, 147.094400 E). Each accession was sown in triplicate in 50 cm rows, with 15 seeds per row and a row spacing of 40 cm, using a randomised block design. Standard agronomic management practises were used to maintain each of the three trials. Disease severity was scored for BYD visual symptoms at heading stage on a 0-5 scale, where 0 = no visible BYD symptoms, 1 = few discoloured leaves scored, 2 = plants had approximately 20% of leaves affected, 3 = 40% of leaves affected, 4 = 60% of leaves affected, and 5 = almost all the plant affected (Fig. 6.1).

### 6.2.4 Genotyping

DNA was extracted from leaf tissue collected at the 2-leaf seedling stage from a single plant per accession and genotyped using the Illumina iSelect 90,000 SNP bead chip assay described in Wang *et al.* (2014). Genome Studio polyploid clustering V1.0 software (Illumina Ltd.) was used to export normalized NormR and Theta values for each accession for SNPs that produced well-separated clusters for unambiguous scoring and had been previously genetically mapped (Wang *et al.*, 2014). SNP genotype calling was performed using a custom PERL script that assigned a genotype to each accession based on the Euclidian distance of the sample data point to the centre of pre-defined clusters having known allelic relationships, considering the standard deviations of the defined clusters. A total of 38,379 SNPs were identified to be polymorphic in the population. The SNP markers with a less

than 90% call rate across samples, a minor allele frequency less than 0.05, or that were redundant were removed. A final number of 4,560 SNPs were used for population structure and kingship analysis.

### 6.2.5 Population Structure and Kinship Analysis

The population structure of the association mapping panel was assessed using all 4,560 SNP markers, which were distributed across the 21 wheat chromosomes, using the software STRUCTURE v2.3.3. (Pritchard *et al.*, 2000). The number of underlying subpopulations was determined from the largest value of the ΔK statistic (Evanno *et al*., 2005). The number of clusters (K) varied from 2 to 8 and 10 iterations were conducted in an admixture model with a 10,000 burning period and 10,000 MCMC (Markov Chain Monte Carlo). K value was the number of clusters when ΔK achieved maximum value (Evanno *et al.*, 2005). SPAGeDi software was used to conduct a kinship analysis (Hardy and Vekemans, 2002). The kinship matrix measured the genetic similarity between individuals.

### 6.2.6 Genome-wide association study

The software TASSEL v3.0 was used to conduct association mapping of BYD resistance in wheat. Information on SNP markers (genotype), population structure, kinship and phenotype traits were imported into TASSEL 3.0. The following models were used for GWAS: (i) Q model, a general linear model (GLM) which sets the Q matrix as a fixed effect; (ii) K model, a mixed linear model (MLM) which sets the kinship matrix as a random effect among genotypes; and (iii) QK model, a MLM which uses both a Q matrix as a fixed effect and a Kinship matrix as a random effect. The Q matrix is a matrix where the number of genotypes and the number of populations were calculated using STRUCTURE software. In the association study, the thresholds were determined as a significant level of $P < 0.001$ ($-\log_{10}(P) > 3$). Manhattan plots were drawn using R software (v2.14.2). Quantile-quantile (Q-Q) plots implemented in TASSEL v3.0 were used to evaluate the fitness and efficiency of these models.

### 6.2.7 Detection of BYD resistant genes through polymerase chain reaction (PCR)

Four markers which are reported to be closely linked to *Bdv2* or *Bdv3* genes were used to assess the presence or absence of the tolerance genes in wheat germplasm. The details of the primer pairs for these markers are listed in Table 6.1. A PCR was performed in 20 µL reaction mixtures containing approximately 15 ng of gDNA, and 0.4 µM of each species-specific forward and reverse primers. The amplification reactions were performed using the PCR system (Bio-Rad T100TM Thermal cycler, USA). The amplification conditions were as follows: initial denaturation at 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s, and a final extension step at 72 °C for 5 min. To verify the PCR results, PCR products were resolved by 1.5% agarose gel electrophoresis. After electrophoresis, the gels were documented under UV using gel documentation system (Bio-Rad, USA).

### 6.2.8 Functional annotation of putative genes in the region of QTL for BYD resistance

To analyse the biological functions of putative genes associated with BYD resistance, we performed a functional annotation of around 2 Mb physical nucleotide interval of significant SNP markers of each QTL. The sequences of significant SNP markers were blasted on https://wheat-urgi.versailles.inra.fr/ and https://urgi.versailles.inra.fr/blast/?dbgroup=wheat_whole_genome_assemblies&program=blastn. Annotated functions in wheat were downloaded from https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Annotations/v1.0/

**Table 6.1** Association List of markers with chromosomal location used for identifying BYD resistance gene in wheat

| **Marker type** | **Primer** | **R gene** | **Premer sequence** | | |
|---|---|---|---|---|---|
| | | | **Forward (5' - 3')** | **Reverse (5' - 3')** | **Reference** |
| SCAR | BYAgi | *Bdv2* | ACT TCA TTG TTG ATC TTG CAT G | CAT GGA TAA TTC AGG GAG CAT TCT G | Stoutjesdijk *et al.* (2001) |
| SCAR | AD2 | *Bdv2* | TGA ACC GCT TCC AGT AAT GGA C | CTG AAC CGC TTC AGC GGT TCA G | Jahier *et al.* (2009) |

| | | | | | |
|---|---|---|---|---|---|
| SSR | Xgwm37 | *Bdv2* | ACT TCA TTG TTG ATC TTG CAT G | CGA CGA ATT CCC AGC TAA AC | Ayala *et al.* (2001) |
| SSR | Bdv3 | *Bdv2/ Bdv3* | CGA CGA ATT CCC AGC TAA ACT AGA CT | CTT AAC TTC ATT GTT GAT CTT A | Kong *et al.* (2009) |

## 6.3 Results

### 6.3.1 BYD resistance of wheat accessions

Visual symptom scoring (VSS) was performed at the heading stage when most of the sensitive varieties (e.g., Revenue, Yu-1) revealed prominent visual symptom. Resistance scores showed a high level of variation among trials and replicates, especially among the susceptible accessions (Suppl. Fig. 6.1). This led to low correlations (even though significant) (Suppl. Fig. 6.2) between trials and low heritability of VVS ($h_B^2 = 0.11$). For examples, Yu-10, a sensitive genotype, ranged from 0 (no symptom) to 5 (very susceptible) across different trials and replicates. However, some resistant lines showed resistance in all the trials/replicates, with no symptom scores being over 2. This variability is not surprising since BYDV infection relies on both aphid spread and the proportion of highly viruliferous aphids in the population, which are extremely variable under field conditions. Thus, it is crucial to obtain phenotypic data from multiple trials. In this study, the average scores of three trials over two years were used for further analysis. Of all the accessions, 2% were resistant (VSS=0-1), 33% moderately resistant (VSS ≥1-2), 61% susceptible (VSS ≥2-3) and 3% highly susceptible (VSS ≥3) to BYDV.


**Fig. 6.1** BYD symptom severity scores in wheat; score 0, whole plant without symptoms; score 1, few leaves showing discoloration; score 2, about 20% leaf area has discoloration, score 3, 40% leaf area shows yellowing; score 4, 60%, leaf area shows yellowing; score 5, most of plant affected.

To determine if any of the resistant genotypes contained *Bdv2* or *Bdv3* genes carried on the group 7 translocations from *Th. intermedium*, several markers linked to the translocations (Zhang *et al.*, 2004; Kong *et al.*, 2009) were used to screen the entire population. Known *Bdv2* containing wheat varieties Mackellar, Manning and Zhong4 (Ayala-Navarrete *et al.*, 2007; Xin *et al.*, 1988) were used as positive controls. Except for these positive controls and XuBYDV, a breeding line from China, all other genotypes in the collection showed a different amplicon size, indicating the absence of *Bdv2* or *Bdv3* genes (Suppl. Fig. 6.3).

### 6.3.2 Association mapping for BYD resistance

The 335 wheat accessions were used to analyse the population structure. To determine the most probable number of subpopulations among all accessions, the largest value of statistic index ΔK was used as an indicator (Evanno *et al.*, 2005). In this study, ΔK reached its highest value when K = 3 (Fig 6.2), suggesting the accessions were comprised of three subpopulations. Details of the subpopulation structure for each of the 335 wheat accessions are shown in Fig. 6.3 and listed in Additional file (Appendix 6.1).

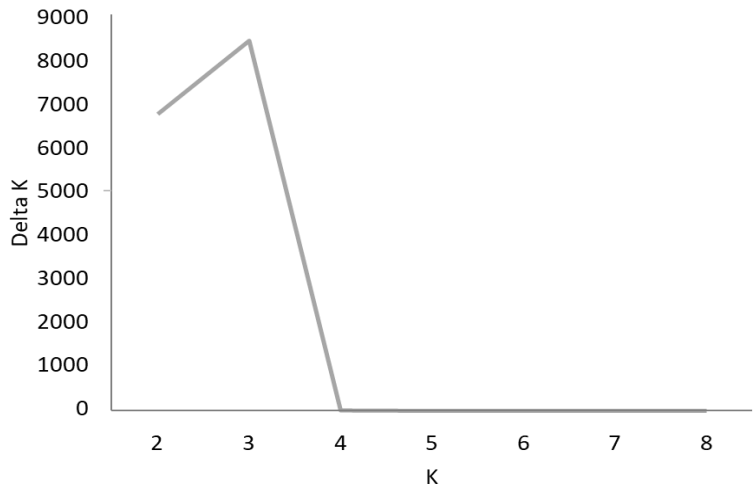



**Fig. 6.2** An estimation of the most probable number of clusters (K), based on 20 independent runs and K ranging from 2 to 8ontrol).

A total of 36 significant marker-trait associations were identified using the Q method. These markers were located on 1A, 2A, 2B, 2D, 3B, 3D, 4A, 5A, 5B, 5D, 6A, 6B, 7A, 7B and 7D (Fig. 6.4a, b & c; Table 6.2) and represented 24 potential QTL (Fig. 6.4a; Table 6.2). Only four significant marker trait associations were

detected with the K method, with the QTL being located on 2A, 2B, 6A and 7A (Fig. 6.4b; Table 6.2). Five significant marker trait associations were detected with the Q+K method, with the QTL being located on 2A, 2B, 6A, 7A and 7B (Fig.6.4c; Table 6.2). Criteria for significant marker-trait association was set for *P*-value <0.001. Markers IWA3520 on 2A (276.89 cM), IWB24938 on 2B (82.22 cM), IWB69770 on 6A (284.1 cM), IWB57703 on 7A (624.47 cM) and IWB65432 on 7B (522.37 cM) showed significant (P<0.001) associations with BYD resistance in at least two methods (Fig. 6.4a, b& c; Table 6.2). The fitness and efficiency assessment of different models by Quantile-Quantile (Q-Q) plot indicated that the observed $-\log_{10}$ (P) values for BYD resistance were closer to expected $-\log_{10}$ (P) values in both the K (Fig. 6.4e) and Q+K (Fig. 6.4f) methods, than those from the Q method (Fig. 6.4d).

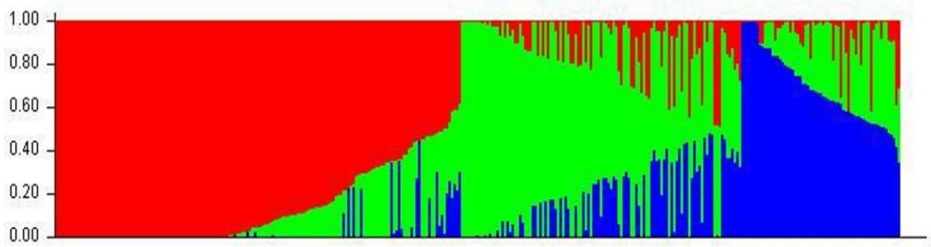



**Fig. 6.3** The population structure of 335 wheat accessions. Three subpopulations (K=3) were produced on genetic diversity detected by 4560 SNP markers, each are presented by a different.

**Table 6.2** Association mapping results for BYDV resistance with the Q method, K method and Q+K method respectively (P<0.01)

| Method | Trait | Chromosome | Position | Marker | *P* | Marker $R^2$ |
|---|---|---|---|---|---|---|
| K (MLM) | BYDV | 2A | 276.8 | IWA3520 | 8.28E-04 | 0.044 |
| | BYDV | 2B | 87.2 | IWB24938 | 3.10E-04 | 0.052 |
| | BYDV | 6A | 284.1 | IWB69770 | 3.40E-05 | 0.074 |
| | BYDV | 7A | 624.4 | IWB57703 | 2.13E-04 | 0.056 |
| Q+K | BYDV | 2A | 276.8 | IWA3520 | 4.78E-04 | 0.051 |
| (MLM) | BYDV | 2B | 87.2 | IWB24938 | 2.64E-04 | 0.055 |
| | BYDV | 6A | 284.1 | IWB69770 | 2.50E-05 | 0.076 |
| | BYDV | 7A | 624.4 | IWB57703 | 2.97E-04 | 0.056 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | BYDV | 7B | 522.3 | IWB65432 | 6.64E-04 | 0.048 |
| Q (GLM) | BYDV | 1A | 219.5 | IWA3884 | 9.28E-04 | 0.043 |
| | BYDV | 1A | 462.6 | IWB24575 | 4.39E-04 | 0.044 |
| | BYDV | 2A | 276.8 | IWB11734 | 4.54E-05 | 0.064 |
| | BYDV | 2A | 276.8 | IWA3520 | 1.75E-05 | 0.065 |
| | BYDV | 2A | 355. | IWB28709 | 4.97E-04 | 0.044 |
| | BYDV | 2B | 87.2 | IWB24938 | 4.81E-04 | 0.045 |
| | BYDV | 2B | 368.5 | IWB54597 | 8.08E-04 | 0.043 |
| | BYDV | 2D | 174.5 | IWB54962 | 1.01E-04 | 0.054 |
| | BYDV | 2D | 177.3 | IWB22279 | 1.09E-04 | 0.053 |
| | BYDV | 2D | 252.8 | IWB16884 | 9.14E-05 | 0.054 |
| | BYDV | 3B | 494.3 | IWB72293 | 1.27E-04 | 0.053 |
| | BYDV | 3D | 276.8 | IWB59242 | 4.84E-04 | 0.044 |
| | BYDV | 3D | 311.1 | IWB73071 | 7.14E-04 | 0.044 |
| | BYDV | 3D | 416.7 | IWB63275 | 9.72E-04 | 0.039 |
| | BYDV | 4A | 589.4 | IWB34365 | 4.19E-04 | 0.048 |
| | BYDV | 5A | 78.1 | IWB28729 | 4.93E-04 | 0.043 |
| | BYDV | 5A | 78.5 | IWB63883 | 6.40E-04 | 0.042 |
| | BYDV | 5A | 335.4 | IWB11870 | 5.00E-04 | 0.045 |
| | BYDV | 5A | 414.1 | IWB25845 | 1.25E-04 | 0.052 |
| | BYDV | 5A | 453.3 | IWB44169 | 5.47E-04 | 0.043 |
| | BYDV | 5B | 180.9 | IWB22443 | 9.14E-05 | 0.054 |
| | BYDV | 5B | 390.6 | IWB25562 | 8.35E-06 | 0.073 |
| | BYDV | 5B | 390.6 | IWB28146 | 2.89E-04 | 0.047 |
| | BYDV | 5B | 392.5 | IWB47520 | 2.74E-04 | 0.048 |
| | BYDV | 5B | 400.8 | IWB33134 | 5.08E-04 | 0.045 |
| | BYDV | 5B | 400.8 | IWA8250 | 7.73E-04 | 0.042 |
| | BYDV | 5D | 185.6 | IWB67931 | 9.52E-05 | 0.057 |
| | BYDV | 6A | 32.3 | IWB72957 | 8.30E-04 | 0.041 |
| | BYDV | 6A | 284.1 | IWB69770 | 3.32E-08 | 0.110 |
| | BYDV | 6B | 218.8 | IWB3623 | 6.95E-04 | 0.041 |
| | BYDV | 6B | 220.9 | IWB59537 | 1.60E-04 | 0.059 |
| | BYDV | 6B | 377.2 | IWB5587 | 2.54E-04 | 0.049 |
| | BYDV | 7A | 135 | IWB9788 | 2.96E-04 | 0.052 |
| | BYDV | 7A | 398 | IWB26913 | 9.21E-04 | 0.039 |
| | BYDV | 7A | 526.3 | IWB34095 | 8.60E-05 | 0.057 |
| | BYDV | 7A | 624.4 | IWB57703 | 9.49E-05 | 0.057 |

| | | | | | |
|---|---|---|---|---|---|
| BYDV | 7A | 625.3 | IWB9343 | 4.43E-04 | 0.049 |
| BYDV | 7B | 427.7 | IWA4750 | 4.98E-04 | 0.044 |
| BYDV | 7B | 482.1 | IWB50373 | 1.49E-04 | 0.052 |
| BYDV | 7B | 522.3 | IWB65432 | 2.62E-04 | 0.049 |
| BYDV | 7B | 547.8 | IWB57016 | 1.27E-04 | 0.056 |
| BYDV | 7D | 298. | IWB15255 | 9.14E-05 | 0.054 |
| BYDV | 7D | 325 | IWB46351 | 5.57E-04 | 0.043 |
| BYDV | 7D | 337.2 | IWB20242 | 2.83E-04 | 0.047 |

*BYDV, *barley yellow dwarf virus* resistance data are averaged of three trials over two growth seasons

The average symptom scores for genotypes carrying different combinations of the resistance alleles is shown in Figure 6.5. Genotypes with resistance alleles generally showed higher tolerance than those without any resistance alleles. Further the effect of the resistance alleles appeared to be additive since the average score decreased in genotypes that carried an increasing number of resistance alleles.

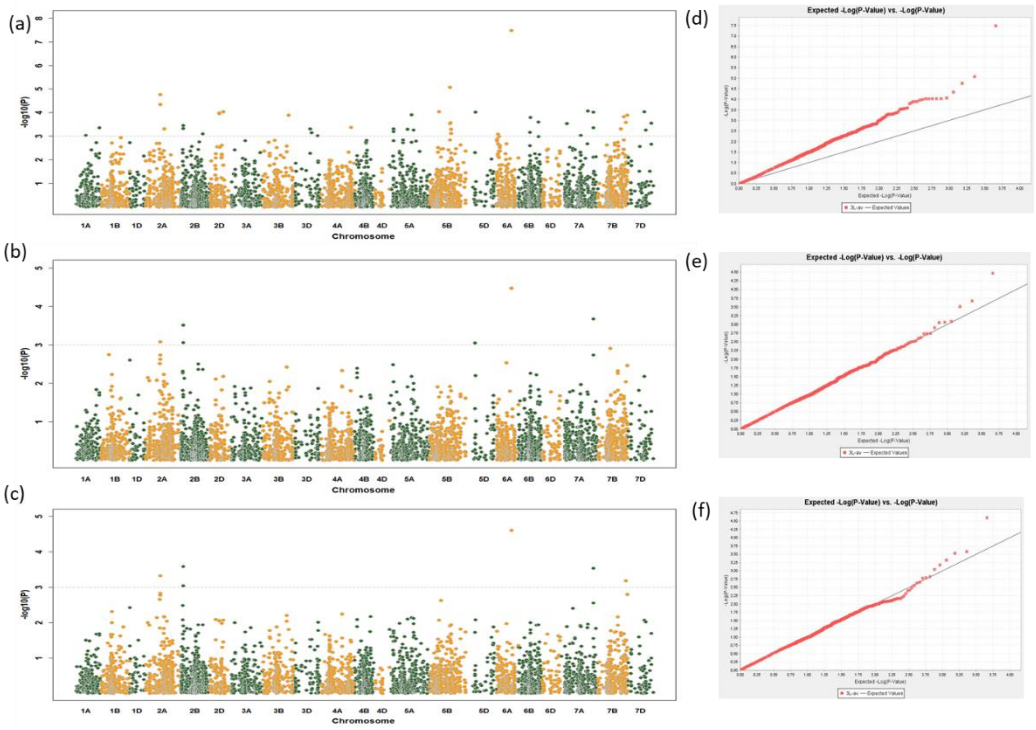



**Fig. 6.4** Manhattan plots and Quantile-quantile (Q-Q) plots for genome wide association study (GWAS) of BYDV resistance in 335 wheat accessions. (a) Manhattan plot in Q method; (b) Manhattan plot in K method; (c) Manhattan plot in Q+K method; (d) Q-Q plot in Q method; (e) Q-Q plot in K method; (f) Q-Q plot in Q+K method. In Manhattan plots, significant association was identified using criterion of –log10 (P) >3 (P < 0.001). Q-Q plots were displayed in marker–trait association analysis. The black line represents

the expected line under the null distribution, while the red symbol in the observed – log10 (P) for BYDV resistance.

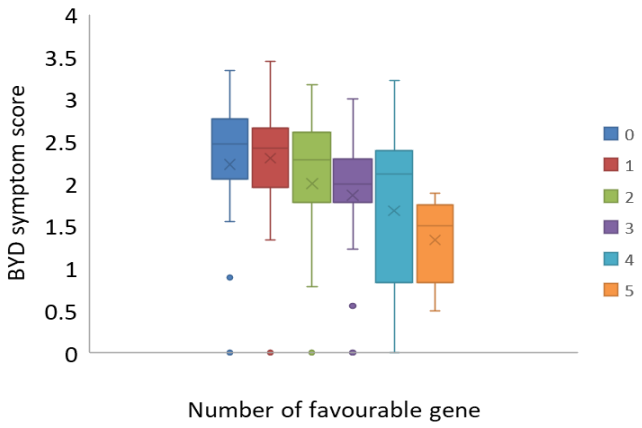
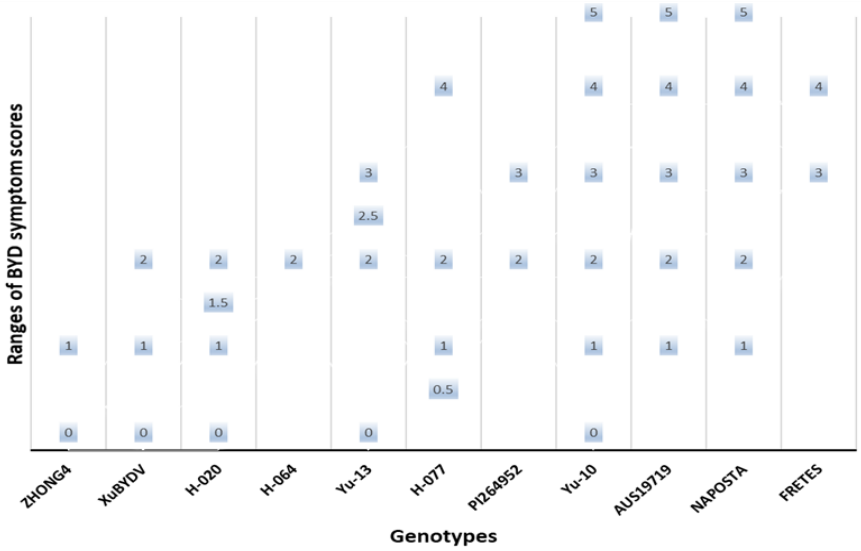
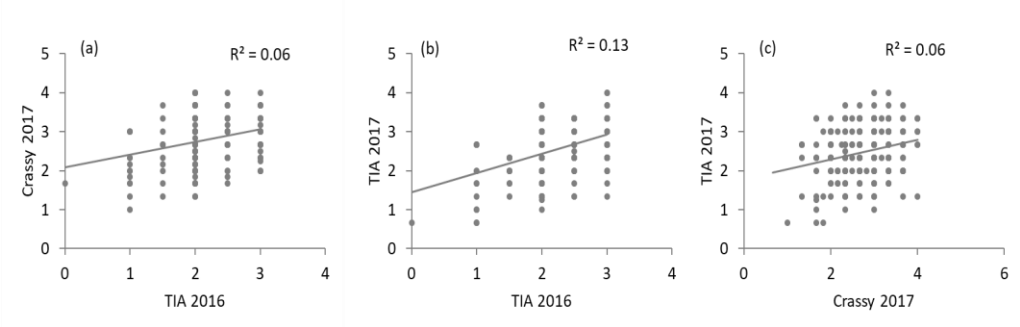


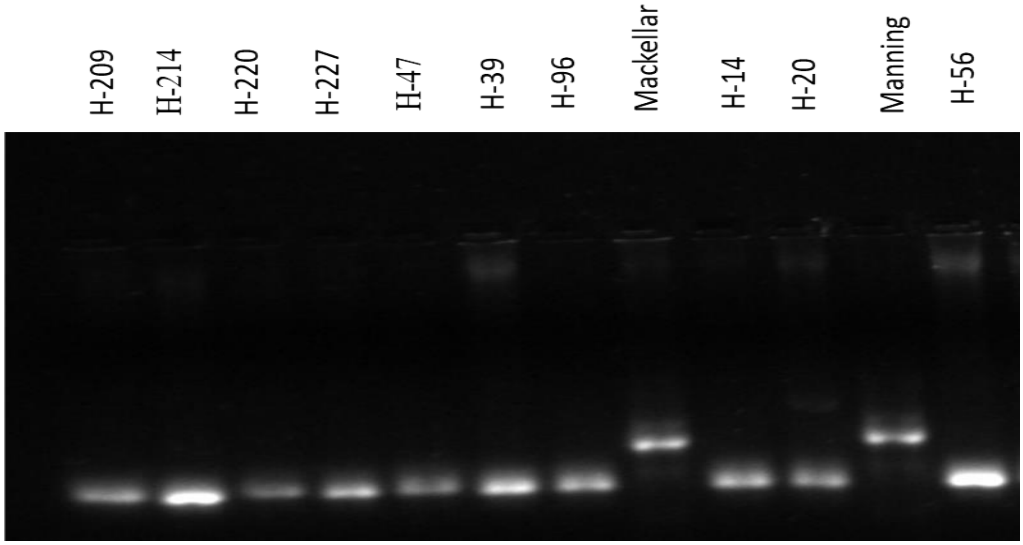

**Fig. 6.5** Five QTL showed an additive effect with the average symptom score (1.33) of wheat accessions combining all five resistance alleles than that of wheat accessions with all susceptible alleles (2.45);0: without any tolerance alleles, 1-4: with 1-4 resistance alleles, 5: with all five resistance alleles.




**Suppl. Fig. 6.1** Distribution of BYD symptom scores of selected genotypes over different trails/replications.




**Suppl. Fig. 6.2** Correlations between different trials for visual symptom scoring.




**Suppl. Fig. 6.3** PCR products of BYDV gene (*Bdv2*) congaing cultivars (Mackellar and Manning) and resistant lines (H-14, H-20, H-39, H-96) amplified with the SSR primer Bdv3.

## 6.4 Discussion

Barley yellow dwarf is one of the most important viral diseases of cereals worldwide and can lead to substantial yield losses that could potentially threaten food security (Strange and Scott, 2005). Available resistances to BYD in wheat are only partially effective but can be pyramided using marker assisted breeding to achieve higher levels of resistance (Francki *et al.*, 2001; Jahier *et al.*, 2009). To further enhance resistance to BYD in common wheat, the identification of new sources of BYD resistance is important. In this study, 335 common wheat accessions were assessed for BYD resistance and a genome wide association study to identify genomic regions for BYD resistance was performed. Five markers were

identified to have consistent and significant associations with BYD resistance using two different GWAS models.

The leaf symptoms found in BYDV-infected plants are commonly used to select BYD resistance in cereal crops (Veškrna *et al.*, 2009; Kosova *et al.*, 2008; Šíp *et al.*, 2006). Most of the genotypes tested in this study showed symptoms of BYDV infection, which was confirmed by detection of the virus using ELISA (data not shown). The scores of the symptoms were used to identify associated QTL. Of the three models used in this study, the Q model showed more associations than the K and Q+K models. This was expected from previous work showing that MLM typically detects fewer QTL than GLM models because the use of both the Q and K matrixes helps to reduce confounding factors such as population structure (Yu and Buckler 2006; Turuspekov *et al.* 2016). Four identical QTL were identified in all the methods. These QTL were located on 2A (276.89 cM), 2B (87.22), 6A (284.1 cM) and 7A (624.47 cM), respectively (Fig.6.4; Table 6.2). An additional QTL on 7B (522.37 cM) was identified in two of the models. By comparing the position of asociated markers with the consensus SNP map, the QTL for BYD resistance on chromosome 2B is at a different position to that identified for BYDV induced dwarfism and biomass reduction in the wheat population Opata × Synthetic (Ayala *et al.*, 2002). The QTL on 6A (284.1 cM) with the nearest marker IWB69770 was located at a different position (long arm) to the reported QTL for yellowing (Ayala *et al.*, 2002). No QTL for BYD resistance was reported by Ayala *et al.* (2002) on chromosome 2A,7A and 7B, which were identified in the current study. These five QTL showed an additive effect with the average visal symptom score of the lines containing resistance alleles of all five QTL being much lower than those with less favourate alleles (Fig. 5).

Few wheat cultivars are reported to have a high level of BYD resistance (Veškrna *et al.*, 2009). The current three BYD resistance genes used in breeding programs, *Bdv2*, *Bdv3* and *Bdv4*, are all on translocations from *Th. intermedium* (intermediate wheatgrass) (Zhang *et al.*, 2009). Zhong 4 is a partial amphiploid between wheat and *Th. intermedium*, 2n=56, with 7 pairs of chromosomes added from *Th. intermedium* (Xin *et al.*, 1991). Zhong 4 is known to have BYD resistance genes on both a group 2 and a group 7 wheatgrass chromsome (Larkin *et al.*, 1995; Wang *et al.*, 2010) whose combined effect is strong resistance (Jahier *et al.*, 2009). Zhong 4

was added to the current study as a resistant control and showed a consistently low level of infection in all trials. BYD resistance was also identified from wheat germplasm with some showing a similar to or even better BYD resistance than Zhong 4. Most of these resistant genotypes are from China which included XuBYDV, H-151, H-20, H-205, H-027, H-023 and H-056. After screening the population with a *Bdv2* or *Bdv3* specific marker, *SSR-Bdv3* (Kong *et al.*, 2009), it was confirmed that none of these resistant genotypes contained the *Bdv2* or *Bdv3* gene. This opens the opportunity of discovering new BYD resitance genes. Further bi-parental populations will be produced to confirm and undertake fine mapping the new QTL for BYD resistance in wheat.

A total of 224 annotated genes were identified in around 10 Mbp of genomic sequence corresponding to the QTL intervals in chromosomes 2A, 2B, 6A, 7A and 7B in Additional file (Appendix 6.2). Among these, eight candidate genes were predicted to relate to plant defense in different species based on published results. Three candidate genes were found for the 2 Mbp QTL interval on chromosome 2B: a receptor kinase-like protein (TraesCS2B01G037300) gene which mediates disease resistance by activating cellular defense response (Song *et al.*, 1995); a subtilisin-like protease (TraesCS2B01G038300) gene which is involved in plant defense response to biotic stress with the manipulation of cell wall, the processing of peptide growth factors and programmed cell death (Schaller *et al.*, 2012); and a glycine-rich protein (TraesCS2B01G038200) gene which has been shown to enhance plant defense mechanism (Mousavi and Hotta, 2005). For the major QTL on chromosome 6A, there were five candidate genes in the 2.4 Mb QTL interval. Among these TraesCS6A01G368200 and TraesCS6A01G368400 encode peroxidases which play a pivotal role in chemical defense mechanisms that control the development of virus disease in many plants (Gonçalves *et al.*; 2013; Hernández *et al.*, 2016; Rai *et al.*, 2010). TraesCS6A01G367000.1 and TraesCS6A01G367000.2 encode bifunctional nucleases involved in basal defense response, participating in abscisic acid-derived callose deposition following infection by a necrotrophic pathogen (LeBrasseur *et al.*, 2002). TraesCS6A01G367800 encodes a leucine-rich repeat protein kinase which acts as as a contributor to basal defense against Fusarium head blight and as an upstream

component of salicylic acid signaling in wheat (Thapa *et al.*, 2018). These candidate genes can be selected as target genes in future study.

## 6.5 Conclusions

This is the first GWAS study that utilize the wheat iSelect 90K SNP array to explore BYD resistance QTL. A total of five significant QTL were identified. Some of the genotypes in the study showed similer or even better resistance to BYD than those genotypes with known resistance *Bdv2* but contained resistance genes different from *Bdv2*. With further characterisation, these lines and the five identified QTL will be useful for breeders to generate combinations with and/or without *Bdv2* to achieve higher levels and more stable BYD resistance.

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# Chapter 8. General Discussion and Conclusion

## 8.1 Selection criterion for BYD resistance

BYD is one of the most serious viral diseases of cereal crops, causing economic losses through reduction in yield when susceptible cultivars are grown. Resistant/tolerant genotypes is the most cost-effective and environmentally sound approach for reducing the damage of BYD disease. Even though genotyping has progressed rapidly, phenotyping remains a bottleneck because it is difficult to find reliable and convenient screening methods for BYD resistance. In this study, visual symptom score, several growth and yield traits have been evaluated for the possibilities of being used as selection criteria for BYD resistance. Virus infection was also confirmed using ELISA and TBIA. Chapin *et al.* (2001) found that 96% of stems with showing visual symptoms tested positive for BYDV-PAV. The study exposed that the amount of infected plants depended on genetic background of plant, and a combination of viruliferous aphids number and inoculation access periods. Higher numbers of viruliferous aphids enhanced visual symptom severity in all susceptible genotypes. According to the species of cereal crops the expression of visual symptom differed following early viral inoculation: in oats leaf discolouration was continuous over time, in wheat at early stages of plant growth no leaf discolouration was observed but symptoms developed at late growth stages, and in barley visual symptoms was decreased over the developmental time. The *Ryd2* gene-containing barley genotype displayed leaf discoloration and reduction in plant height but showed no significant yield reductions. This indicates that, yield analyses are needed for evaluating BYDV-PAV tolerance. For wheat, it is essential to assess BYD resistance through ELISA/TBIA tests and shoot biomass at early stage. However, leaf symptoms in BYDV-infected wheat plants can be scored for selecting BYD resistance in in heading stage

## 8.2 Morphological, physiological, biochemical and histological response under BYDV stress

To understand the mechanism of BYD resistance genotypes, we observed the biochemical and ultrastructural alterations in susceptible and resistant wheat and barley genotypes. The root elongation was affected in wheat and barley genotypes

differing in their response to BYDV. Screening of root traits at early plant growth stage can be used as a proxy for mature stages (Comas *et al.*, 2013). Water and nutrient absorption process in plant severely restricted due to reduction in root length, which can lead to the reduced of shoot development. Virus infected plants showed a reduction in chlorophyll content, resulting in leaf chlorosis. Hence, this reduction of photosynthesis capacity led to reduced biomass production.

The photosynthetic system is the physiological basis of crop growth and yield (Sun *et al.*, 2009). Reduction in photosynthetic rate due to virus infection is associated with physical damage to the chloroplast structure and the deterioration of its membranes (Fraser and Fraser, 1987). BYDV infection caused significant reductions in photosynthesis of susceptible wheat genotypes and showed higher negative correlation with symptom score likely because virus infection can reduce green leaf areas or cause transport blockage and accumulation of photosynthate in infected leaves. This abnormal accumulation may inhibit further photosynthesis by disrupting the structure of chloroplast, reducing diffusion of $CO_2$ or availability of light, and may be emphasized by phloem necrosis (Esau *et al.*, 1967). During photosynthesis, stomata play a critical role in $CO_2$ assimilation and C fixation that ultimately contribute to increased plant biomass and grain yield (Hetherington and Woodward, 2003). Chlorophyll fluorescence (Fv/Fm ratio) provides basic information regarding photosynthetic apparatus (Rapacz and Hura, 2004), suggesting virus infection destroys functional photosynthetic reaction leading to chlorophyll degradation (lower SPAD values). Therefore, BYD-resistant genotype, which carries the resistance gene, showed good performance in physiological and yield contributing parameters with low levels of infection rates.

Plants infected by the virus showed strong metabolic and ultrastructural alterations even when no visible symptoms occurred (Yan *et al.*, 2008). In virus-infected resistant genotypes, the total phenolic content was significantly increased. In plant cell walls, deposition of phenolics may be a possible mechanism of virus resistance. This might play a significant role by increasing mechanical strength of host cell through lignification of cell wall as lignin precursors. Foliar sugar levels were increased in virus infected plants. This was accompanied by enhanced respiration, which may lead to reduce plant biomass (Shalitin and Wolf, 2000). BYDV infected

plants had a larger accumulation of reducing sugar in susceptible barley and wheat, possibly due to the disturbance of normal phloem transport or phloem loading. BYDV is a systemic virus and it replicates within the plant phloem tissue (Irwin and Thresh, 1990). In vasculature samples, BYDV particles are found exclusively. Any restriction to phloem tissues will impact virus distribution. Although vasculature damage remains to be quantified in BYDV infected plants, we may speculate that accruing viral load in the phloem leads to extensive damage to the vasculature, and inhibit sugar transport, which can reduce plant growth and biomass. The increasing amount of sugar in infected leaves may also inhibit photosynthesis, further inhibiting plant growth. The ability to respond to virus infection with increasing phenolic contents in resistant genotypes may be a response to the *Bdv2* and *Ryd2* genes, thus, limiting viral load and the cascade of pathological events.

## 8.3 QTL controlling BYD resistance

In this study, one DH population and one natural population with diverse germplasm were used for detecting QTL through bi-parental QTL mapping and genome wide association mapping. In the natural population with more than 300 geographically diverse wheat germplasm collections, four novel QTL on chromosomes 2A, 2B, 6A and 7A QTL for BYD resistance were consistently detected in all GLM (Q- model) and MLM (K model and Q+K model). An additional QTL on 7B (522.37 cM) was identified in two of the models (Q and K). This was the first GWAS study on BYD resistance in wheat accessions. Several wheat genotypes showed consistent resistance in different field trials. All these genotypes did not contain previously reported BYD resistance gene. Therefore, these genotypes will be used in our further research to confirm the QTL identified in this research or map new QTL for BYD resistance.

The DH population from the cross of XuBYDV and H-120 were screened for BYD resistance in two different years. This population was genotyped using a high-density wheat SNP array containing iSelect 90K SNPs. Disease symptom of BYDV infected plants was visually assessed at heading stage. BYDV infection was tested by Tissue bolt immunoassay (TBIA). Both visual scores and TBIA results were used for QTL analysis. A major QTL for BYD tolerance was detected on chromosome 7A from both years' trials. No QTL was reported for BYD tolerance

on 7A of the QTL identified by all the trails in both traits in the current study. In general, chromosome 7 may be the special interest for BYD tolerance because in both *Thinopyrum intermedium* and wheat (*Triticum aestivum*) tolerance has been assigned to this chromosome (Singh *et al.*, 1993; Larkin *et al.*, 1995). Two other significant QTL with less significance compared to the major QTL were also identified in this study. These two QTL were located on chromosome 5A and 6A, respectively. All these new QTL will be useful in breeding programs for pyramiding BYD resistant genes.

## 8.4 General conclusion and recommendations

In conclusion, five significant QTL for BYD resistance were identified in genome wide association studies. They are located on chromosome 2A, 2B, 6A, 7A and 7B. These five QTL have not been reported before. Further bi-parental populations will be produced to confirm and undertake fine mapping the new QTL for BYD resistance in wheat. In DH population, a total of three new QTL for BYDV resistance were detected on chromosome 5A, 6A and 7A. The development of diagnostic marker will facilitate map-based gene cloning and promoting the efficiency for MAS in breeding BYD resistant wheat. Once diagnostic marker that represent BYD resistance have been developed, resistance genes can be introgressed into elite genotype through MAS and ultimate QTL pyramiding.

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# Appendix

**Appendix 6.1** The value of population structure of 335 genotypes. Each genotype belongs to the population with the highest value calculated by STRUCTURE software

| Genotype | Q1 | Q2 | Q3 |
|---|---|---|---|
| 073/44 | 0.173 | 0.826 | 0.001 |
| 92FS-16 | 0.022 | 0.946 | 0.032 |
| AEGYLOPS142 | 0.002 | 0.994 | 0.004 |
| AFGHANISTAN | 0.002 | 0.836 | 0.163 |
| AHGAF | 0.002 | 0.852 | 0.147 |
| ALBIDUM24 | 0.006 | 0.518 | 0.476 |
| Al-wheat | 0.073 | 0.575 | 0.352 |
| ARTEMOVKA | 0.007 | 0.785 | 0.208 |
| AUS19392 | 0.008 | 0.719 | 0.272 |
| AUS19393 | 0.031 | 0.693 | 0.275 |
| AUS19394 | 0.183 | 0.187 | 0.63 |
| AUS19395 | 0.003 | 0.753 | 0.244 |
| AUS19396 | 0.067 | 0.91 | 0.024 |
| AUS19397 | 0.007 | 0.978 | 0.016 |
| AUS19398 | 0.027 | 0.97 | 0.002 |
| AUS19399 | 0.028 | 0.97 | 0.002 |
| AUS19400 | 0.005 | 0.985 | 0.01 |
| Aus19401 | 0.005 | 0.713 | 0.282 |
| AUS19402 | 0.33 | 0.666 | 0.004 |
| AUS19403 | 0.698 | 0.301 | 0.001 |
| AUS19719 | 0.07 | 0.582 | 0.348 |
| Aus19720 | 0.002 | 0.996 | 0.002 |
| B-T-17 | 0.001 | 0.362 | 0.637 |
| B-T-35 | 0.001 | 0.48 | 0.519 |
| B-T-38 | 0.003 | 0.695 | 0.302 |
| B-T-51 | 0.001 | 0.731 | 0.268 |
| BUKOVINKA | 0.003 | 0.851 | 0.145 |
| CAZ53 | 0.705 | 0.293 | 0.002 |
| E.M.S.SUMMI | 0.076 | 0.922 | 0.003 |
| EXITOB | 0.018 | 0.698 | 0.284 |

| | | | |
|---|---|---|---|
| FERRUGINEUM | 0.166 | 0.464 | 0.369 |
| FRETES | 0.135 | 0.864 | 0.001 |
| FRETES | 0.136 | 0.863 | 0.001 |
| FRETES | 0.138 | 0.861 | 0.001 |
| Glover | 0.048 | 0.597 | 0.355 |
| H-001 | 0.095 | 0.524 | 0.381 |
| H-002 | 0.003 | 0.352 | 0.645 |
| H-003 | 0.382 | 0.315 | 0.302 |
| H-004 | 0.889 | 0.11 | 0.001 |
| H-005 | 0.852 | 0.147 | 0.001 |
| H-006 | 0.909 | 0.09 | 0.001 |
| H-007 | 0.839 | 0.161 | 0.001 |
| H-008 | 0.207 | 0.789 | 0.004 |
| H-009 | 0.853 | 0.146 | 0 |
| H-010 | 0.92 | 0.079 | 0.001 |
| H-011 | 0.681 | 0.316 | 0.004 |
| H-012 | 0.999 | 0 | 0 |
| H-013 | 0.001 | 0.998 | 0.001 |
| H-014 | 0.008 | 0.446 | 0.546 |
| H-015 | 0.999 | 0 | 0 |
| H-016 | 0.999 | 0 | 0 |
| H-017 | 0.999 | 0.001 | 0 |
| H-019 | 0.999 | 0.001 | 0 |
| H-020 | 1 | 0 | 0 |
| H-021 | 0.998 | 0 | 0.001 |
| H-022 | 0.64 | 0.32 | 0.04 |
| H-023 | 0.999 | 0 | 0 |
| H-024 | 1 | 0 | 0 |
| H-025 | 0.999 | 0 | 0 |
| H-027 | 0.999 | 0 | 0 |
| H-028 | 0.015 | 0.983 | 0.002 |
| H-030 | 0.999 | 0 | 0 |
| H-031 | 0.516 | 0.181 | 0.303 |
| H-033 | 0.125 | 0.462 | 0.413 |
| H-034 | 0.081 | 0.405 | 0.514 |
| H-037 | 0.48 | 0.512 | 0.008 |

| | | | |
|---|---|---|---|
| H-038 | 0.278 | 0.395 | 0.327 |
| H-039 | 0.226 | 0.431 | 0.343 |
| H-040 | 0.349 | 0.648 | 0.002 |
| H-041 | 0.999 | 0 | 0 |
| H-042 | 0.65 | 0.002 | 0.348 |
| H-043 | 0.411 | 0.37 | 0.22 |
| H-044 | 0.999 | 0 | 0 |
| H-045 | 0.996 | 0.002 | 0.001 |
| H-046 | 0.996 | 0.002 | 0.001 |
| H-047 | 1 | 0 | 0 |
| H-048 | 1 | 0 | 0 |
| H-049 | 0.999 | 0 | 0 |
| H-051 | 0.999 | 0.001 | 0.001 |
| H-052 | 0.999 | 0 | 0.001 |
| H-053 | 0.999 | 0 | 0.001 |
| H-054 | 0.998 | 0 | 0.002 |
| H-055 | 0.999 | 0 | 0 |
| H-056 | 0.999 | 0.001 | 0 |
| H-057 | 0.997 | 0 | 0.003 |
| H-058 | 0.999 | 0 | 0.001 |
| H-059 | 0.999 | 0 | 0 |
| H-060 | 0.998 | 0 | 0.001 |
| H-061 | 0.999 | 0 | 0.001 |
| H-062 | 0.999 | 0 | 0.001 |
| H-063 | 0.641 | 0.002 | 0.357 |
| H-064 | 0.999 | 0 | 0 |
| H-065 | 0.999 | 0 | 0 |
| H-066 | 0.974 | 0.004 | 0.022 |
| H-067 | 0.031 | 0.719 | 0.25 |
| H-068 | 0.001 | 0 | 0.999 |
| H-069 | 0.648 | 0.335 | 0.018 |
| H-070 | 0.527 | 0.468 | 0.004 |
| H-071 | 0.415 | 0.001 | 0.583 |
| H-073 | 0.008 | 0.291 | 0.701 |
| H-074 | 0.001 | 0 | 0.999 |
| H-075 | 0.419 | 0.057 | 0.524 |

| | | | |
|---|---|---|---|
| H-076 | 0.999 | 0 | 0 |
| H-077 | 0.888 | 0.111 | 0.001 |
| H-078 | 0.001 | 0.998 | 0.001 |
| H-079 | 0.421 | 0.398 | 0.181 |
| H-080 | 0.997 | 0.003 | 0.001 |
| H-081 | 0.413 | 0.586 | 0.001 |
| H-082 | 0.001 | 0.991 | 0.008 |
| H-083 | 0.036 | 0.387 | 0.577 |
| H-084 | 0.127 | 0.541 | 0.333 |
| H-085 | 0.045 | 0.594 | 0.361 |
| H-086 | 0.004 | 0.49 | 0.506 |
| H-087 | 0.003 | 0.993 | 0.005 |
| H-088 | 0.005 | 0.545 | 0.45 |
| H-089 | 0.052 | 0.491 | 0.458 |
| H-090 | 0.002 | 0.317 | 0.681 |
| H-091 | 0.01 | 0.004 | 0.986 |
| H-092 | 0.023 | 0.35 | 0.627 |
| H-093 | 0.008 | 0.115 | 0.878 |
| H-094 | 0.001 | 0 | 0.999 |
| H-095 | 0.148 | 0.268 | 0.584 |
| H-096 | 0.508 | 0.386 | 0.106 |
| H-097 | 0.497 | 0.482 | 0.021 |
| H-098 | 0.008 | 0.988 | 0.004 |
| H-099 | 0.011 | 0.911 | 0.077 |
| H-100 | 0.041 | 0.901 | 0.058 |
| H-101 | 0.005 | 0.746 | 0.249 |
| H-102 | 0.16 | 0.838 | 0.003 |
| H-103 | 0.034 | 0.611 | 0.355 |
| H-104 | 0.001 | 0.52 | 0.479 |
| H-105 | 0.028 | 0.706 | 0.266 |
| H-106 | 0.101 | 0.893 | 0.007 |
| H-107 | 0.999 | 0 | 0.001 |
| H-108 | 0.999 | 0 | 0.001 |
| H-109 | 0.999 | 0 | 0 |
| H-110 | 0.774 | 0.001 | 0.225 |
| H-111 | 0.999 | 0 | 0 |

| | | | |
|---|---|---|---|
| H-112 | 0.314 | 0.342 | 0.344 |
| H-113 | 0.412 | 0.341 | 0.247 |
| H-114 | 0.001 | 0.162 | 0.837 |
| H-115 | 0.04 | 0.436 | 0.524 |
| H-116 | 1 | 0 | 0 |
| H-117 | 0.999 | 0.001 | 0.001 |
| H-118 | 0.999 | 0.001 | 0 |
| H-119 | 0.582 | 0.374 | 0.044 |
| H-120 | 0.563 | 0.433 | 0.004 |
| H-121 | 0.444 | 0.554 | 0.002 |
| H-122 | 0.391 | 0.539 | 0.069 |
| H-125 | 0.304 | 0.692 | 0.005 |
| H-126 | 0.058 | 0.196 | 0.745 |
| H-127 | 0.052 | 0.201 | 0.747 |
| H-129 | 0.552 | 0.001 | 0.447 |
| H-130 | 0.484 | 0.515 | 0.001 |
| H-131 | 0.199 | 0.404 | 0.397 |
| H-132 | 0.004 | 0.326 | 0.669 |
| H-133 | 0.091 | 0.45 | 0.459 |
| H-135 | 0.089 | 0.432 | 0.478 |
| H-136 | 0.002 | 0.003 | 0.995 |
| H-137 | 0.007 | 0.207 | 0.785 |
| H-138 | 0.357 | 0.025 | 0.618 |
| H-141 | 0.151 | 0.542 | 0.307 |
| H-142 | 0.005 | 0.408 | 0.588 |
| H-143 | 0.467 | 0.273 | 0.26 |
| H-144 | 0.999 | 0 | 0 |
| H-145 | 0.954 | 0.045 | 0.001 |
| H-146 | 0.939 | 0.06 | 0.001 |
| H-147 | 0.999 | 0 | 0 |
| H-148 | 0.863 | 0.137 | 0 |
| H-149 | 0.999 | 0.001 | 0 |
| H-150 | 1 | 0 | 0 |
| H-151 | 1 | 0 | 0 |
| H-152 | 1 | 0 | 0 |
| H-153 | 0.782 | 0.108 | 0.11 |

| | | | |
|---|---|---|---|
| H-154 | 0.956 | 0.022 | 0.023 |
| H-157 | 0.998 | 0.001 | 0.002 |
| H-159 | 0.997 | 0.002 | 0.001 |
| H-160 | 1 | 0 | 0 |
| H-161 | 0.903 | 0.097 | 0.001 |
| H-163 | 0.928 | 0.059 | 0.013 |
| H-164 | 0.957 | 0.042 | 0.001 |
| H-165 | 0.969 | 0.028 | 0.003 |
| H-167 | 0.995 | 0.004 | 0.001 |
| H-168 | 0.999 | 0.001 | 0 |
| H-169 | 0.984 | 0.015 | 0.001 |
| H-170 | 0.707 | 0.068 | 0.225 |
| H-171 | 0.999 | 0 | 0 |
| H-172 | 0.999 | 0 | 0 |
| H-176 | 0.496 | 0.304 | 0.201 |
| H-177 | 0.87 | 0.129 | 0.001 |
| H-178 | 0.888 | 0.111 | 0.001 |
| H-179 | 0.78 | 0.22 | 0.001 |
| H-180 | 0.94 | 0.058 | 0.002 |
| H-181 | 0.921 | 0.078 | 0.001 |
| H-183 | 0.894 | 0.105 | 0.001 |
| H-184 | 0.877 | 0.122 | 0.001 |
| H-185 | 0.899 | 0.101 | 0.001 |
| H-186 | 0.999 | 0 | 0 |
| H-187 | 0.761 | 0.238 | 0.001 |
| H-189 | 0.986 | 0.013 | 0.002 |
| H-191 | 0.391 | 0.194 | 0.416 |
| H-192 | 0.034 | 0.142 | 0.824 |
| H-193 | 0.835 | 0.164 | 0.001 |
| H-194 | 0.904 | 0.095 | 0.001 |
| H-195 | 0.849 | 0.15 | 0.001 |
| H-196 | 0.86 | 0.139 | 0.001 |
| H-197 | 0.862 | 0.138 | 0.001 |
| H-199 | 0.806 | 0.194 | 0.001 |
| H-200 | 0.686 | 0.312 | 0.001 |
| H-201 | 0.81 | 0.189 | 0.001 |

| | | | |
|---|---|---|---|
| H-202 | 0.008 | 0.468 | 0.524 |
| H-203 | 0.07 | 0.926 | 0.004 |
| H-204 | 0.187 | 0.781 | 0.031 |
| H-205 | 0.556 | 0.169 | 0.276 |
| H-207 | 0.052 | 0.169 | 0.779 |
| H-208 | 0.021 | 0.302 | 0.678 |
| H-209 | 0.002 | 0.459 | 0.539 |
| H-210 | 0.034 | 0.492 | 0.473 |
| H-211 | 0.001 | 0.727 | 0.272 |
| H-212 | 0.324 | 0.567 | 0.108 |
| H-213 | 0.4 | 0.576 | 0.024 |
| H-214 | 0.532 | 0.285 | 0.183 |
| H-215 | 0.103 | 0.153 | 0.743 |
| H-216 | 0.201 | 0.245 | 0.554 |
| H-217 | 0.882 | 0.116 | 0.002 |
| H-218 | 0.997 | 0.002 | 0.001 |
| H-220 | 0.009 | 0.555 | 0.436 |
| H-221 | 0.003 | 0.222 | 0.775 |
| H-222 | 0.998 | 0.001 | 0.001 |
| H-223 | 0.999 | 0.001 | 0 |
| H-224 | 0.997 | 0.002 | 0.001 |
| H-225 | 0.999 | 0 | 0 |
| H-227 | 0.648 | 0.323 | 0.029 |
| H-228 | 0.999 | 0.001 | 0 |
| H-229 | 0.906 | 0.093 | 0.001 |
| H-230 | 0.998 | 0.001 | 0.001 |
| H-231 | 0.865 | 0.135 | 0.001 |
| H-232 | 0.899 | 0.1 | 0.001 |
| H-233 | 0.999 | 0 | 0 |
| H-234 | 0.999 | 0 | 0 |
| H-235 | 0.001 | 0 | 0.999 |
| H-236 | 0.804 | 0.191 | 0.004 |
| H-237 | 0.001 | 0.599 | 0.401 |
| H-238 | 0.952 | 0.047 | 0.001 |
| H-239 | 0.992 | 0.006 | 0.002 |
| H-240 | 0.934 | 0.061 | 0.005 |

| | | | |
|---|---|---|---|
| H-241 | 0.977 | 0.022 | 0.001 |
| H-242 | 0.982 | 0.018 | 0 |
| H-243 | 0.706 | 0.293 | 0.001 |
| H-246 | 0.53 | 0.47 | 0.001 |
| H-247 | 0.052 | 0.66 | 0.288 |
| H-249 | 0.359 | 0.64 | 0.001 |
| H-250 | 0.617 | 0.381 | 0.002 |
| H-251 | 0.001 | 0.831 | 0.167 |
| H-252 | 0.002 | 0.807 | 0.191 |
| H-253 | 0.002 | 0.822 | 0.176 |
| Hartog | 0.05 | 0.591 | 0.359 |
| HB88I-172 | 0.049 | 0.82 | 0.131 |
| IG43428 | 0.001 | 0.998 | 0.002 |
| KARAGAN | 0.678 | 0.321 | 0.001 |
| KAZAKHSTANS | 0.309 | 0.69 | 0.001 |
| KHARCHIA65 | 0.715 | 0.284 | 0.001 |
| KOPARA73 | 0.06 | 0.718 | 0.221 |
| KORDCLPLUS | 0.078 | 0.678 | 0.243 |
| KZYL-SARK | 0.588 | 0.412 | 0 |
| LU26S | 0.214 | 0.592 | 0.194 |
| Mace | 0.095 | 0.431 | 0.474 |
| MAHONDEMIAS | 0.061 | 0.794 | 0.145 |
| NAJAH | 0.084 | 0.348 | 0.568 |
| NAPOSTA | 0.062 | 0.806 | 0.132 |
| ONOHOISKAJA | 0.003 | 0.991 | 0.005 |
| PALESTINE8 | 0.191 | 0.809 | 0.001 |
| PI178012 | 0.001 | 0 | 0.999 |
| PI178704 | 0.172 | 0.146 | 0.682 |
| PI180988 | 0.668 | 0.331 | 0.001 |
| PI264952 | 0.182 | 0.817 | 0.001 |
| POBEDA | 0.101 | 0.893 | 0.005 |
| PretoAmarel | 0.296 | 0.7 | 0.004 |
| Revenue | 0.001 | 0.537 | 0.462 |
| SEAGULL | 0.004 | 0.923 | 0.073 |
| SELOFHOURAN | 0.196 | 0.803 | 0.001 |
| SELOFHOURAN | 0.196 | 0.803 | 0.001 |

| | | | |
|---|---|---|---|
| SKALA | 0.065 | 0.866 | 0.069 |
| SOCIEDADNAC | 0.013 | 0.355 | 0.632 |
| SONNENWEIZE | 0.169 | 0.731 | 0.1 |
| SURHAK5688 | 0.547 | 0.451 | 0.001 |
| SURHAKMESTN | 0.543 | 0.456 | 0.001 |
| SW9550101 | 0.948 | 0.049 | 0.003 |
| SW9550192 | 0.986 | 0.006 | 0.009 |
| SW9550213 | 0.996 | 0.003 | 0.001 |
| SW9550292 | 0.999 | 0 | 0 |
| TAJAZNAJA4 | 0.235 | 0.703 | 0.062 |
| TURCICUM1 | 0.656 | 0.343 | 0.001 |
| VYS | 0.001 | 0.814 | 0.184 |
| W5013EY-11- | 0.01 | 0.889 | 0.101 |
| W5228HZ-1 | 0.018 | 0.354 | 0.628 |
| W5924DT-34 | 0.016 | 0.781 | 0.202 |
| XuBYDV | 0.152 | 0.482 | 0.366 |
| WL-wheat | 0.756 | 0.013 | 0.231 |
| X-165 | 0.978 | 0.021 | 0.001 |
| X-253 | 0.621 | 0.378 | 0.001 |
| X-254 | 0.71 | 0.29 | 0 |
| X-255 | 0.535 | 0.464 | 0.001 |
| X-256 | 0.489 | 0.511 | 0.001 |
| X-257 | 0.51 | 0.437 | 0.052 |
| X-258 | 0.672 | 0.328 | 0.001 |
| X-259 | 0.655 | 0.345 | 0.001 |
| X-26 | 0.999 | 0 | 0 |
| X-260 | 0.994 | 0.006 | 0.001 |
| X-261 | 0.103 | 0.012 | 0.885 |
| X-262 | 0.017 | 0.331 | 0.652 |
| X-A | 0.009 | 0.32 | 0.671 |
| X-B | 0.224 | 0.775 | 0.001 |
| X-C | 0.208 | 0.79 | 0.001 |
| X-D | 0.187 | 0.667 | 0.145 |
| X-E | 0.16 | 0.835 | 0.006 |
| X-F | 0.004 | 0.587 | 0.409 |
| Yang17 | 0.018 | 0.168 | 0.814 |

| | | | |
|---|---|---|---|
| Yannong15 | 0.002 | 0.436 | 0.562 |
| Yannong19 | 0.002 | 0.567 | 0.431 |
| Yrody1006 | 0.665 | 0.335 | 0.001 |
| Yu-01 | 0.177 | 0.547 | 0.276 |
| Yu-02 | 0.004 | 0.2 | 0.796 |
| Yu-03 | 0.102 | 0.008 | 0.89 |
| Yu-04 | 0.003 | 0.121 | 0.876 |
| Yu-05 | 0.09 | 0.073 | 0.837 |
| Yu-06 | 0.119 | 0.167 | 0.714 |
| Yu-07 | 0.002 | 0.464 | 0.534 |
| Yu-08 | 0.152 | 0.285 | 0.563 |
| Yu-09 | 0.003 | 0.12 | 0.877 |
| Yu-10 | 0.004 | 0.483 | 0.513 |
| Yu-11 | 0.017 | 0.27 | 0.713 |
| Yu-12 | 0.016 | 0.574 | 0.41 |
| Yu-13 | 0.031 | 0.132 | 0.836 |
| Zhong4 | 0.002 | 0.824 | 0.174 |

**Appendix 6.2** Results of the putative candidate genes associated with BYD resistance on chromosome 2A, 2B, 6A, 7A and 7B. Yellow: candidate genes

| Annotated gene ID | Physical position | Putative function |
|---|---|---|
| TraesCS2A01G093700.4 | chr2A:47830215..47833893 | Protein phosphatase 2C-like |
| TraesCS2A01G093800.1 | chr2A:47837621..47839173 | Pleckstrin homology domain-containing family A member 8 |
| TraesCS2A01G093900.1 | chr2A:47875058..47876543 | F-box domain containing protein-like |
| TraesCS2A01G094000.1 | chr2A:47905745..47912978 | PHD and RING finger domain-containing protein 1 |
| TraesCS2A01G094100.1 | chr2A:47913592..47914354 | DUF3511 domain protein, putative (DUF3511) |
| TraesCS2A01G094200.1 | chr2A:47917346..47919716 | Ribosomal protein L19 |
| TraesCS2A01G094300.1 | chr2A:48197649..48200443 | Ta11-like non-LTR retrotransposon |
| TraesCS2A01G094400.1 | chr2A:48221991..48222305 | Retrotransposon protein, putative, unclassified |
| TraesCS2A01G094500.1 | chr2A:48249835..48251371 | Hexosyltransferase |
| TraesCS2A01G094600.1 | chr2A:48259086..48259307 | cytochrome P450, family 87, subfamily A, polypeptide 2 |
| TraesCS2A01G094700.1 | chr2A:48346043..48347600 | Hexosyltransferase |
| TraesCS2A01G094800.1 | chr2A:48393580..48400644 | BRCT domain-containing protein |
| TraesCS2A01G094900.1 | chr2A:48405172..48405879 | Defensin |
| TraesCS2A01G094900.2 | chr2A:48405172..48405879 | Defensin |
| TraesCS2A01G095000.1 | chr2A:48456275..48456707 | Defensin |
| TraesCS2A01G095100.1 | chr2A:48622979..48623242 | ATP-dependent caseinolytic (Clp) protease/crotonase family protein |
| TraesCS2A01G095200.1 | chr2A:48658229..48661328 | DnaJ |
| TraesCS2A01G095300.1 | chr2A:48854801..48865655 | Protein kinase |
| TraesCS2A01G095400.1 | chr2A:48865672..48866440 | Multidrug resistance protein ABC transporter family protein, putative |

| | | |
|---|---|---|
| TraesCS2A01G095500.1 | chr2A:48866777..48877173 | Lon protease homolog |
| TraesCS2A01G095600.1 | chr2A:49171612..49183460 | Glutamyl endopeptidase, putative |
| TraesCS2A01G095600.2 | chr2A:49172259..49183460 | Dipeptidyl-peptidase, putative |
| TraesCS2A01G095700.1 | chr2A:49220989..49222345 | Nicotianamine synthase |
| TraesCS2A01G095800.1 | chr2A:49270239..49272707 | F-box protein family-like |
| TraesCS2A01G095900.1 | chr2A:49306990..49312476 | Pyridoxal phosphate (PLP)-dependent transferases superfamily protein |
| TraesCS2A01G096000.1 | chr2A:49845101..49851814 | Aldehyde dehydrogenase |
| TraesCS2B01G034100.1 | chr2B:16772083..16774207 | Glycosyltransferase |
| TraesCS2B01G034200.1 | chr2B:16813576..16816504 | Receptor-like protein kinase |
| TraesCS2B01G034200.2 | chr2B:16813576..16816571 | Receptor-like protein kinase |
| TraesCS2B01G034300.1 | chr2B:16825642..16828496 | receptor kinase 1 |
| TraesCS2B01G034300.2 | chr2B:16825671..16828473 | Receptor-like protein kinase |
| TraesCS2B01G034400.1 | chr2B:16828794..16829412 | Defensin |
| TraesCS2B01G034500.1 | chr2B:16847634..16848199 | Defensin |
| TraesCS2B01G034600.1 | chr2B:16854910..16857737 | receptor kinase 1 |
| TraesCS2B01G034600.2 | chr2B:16854910..16857737 | receptor kinase 1 |
| TraesCS2B01G034600.3 | chr2B:16854910..16857735 | receptor kinase 1 |
| TraesCS2B01G034700.1 | chr2B:16867978..16872120 | receptor kinase 1 |
| TraesCS2B01G034700.2 | chr2B:16867978..16872120 | Receptor-like protein kinase |
| TraesCS2B01G034800.1 | chr2B:16891581..16894636 | GDSL esterase/lipase |
| TraesCS2B01G034900.1 | chr2B:16895460..16898470 | Carboxyl methyltransferase |
| TraesCS2B01G035000.1 | chr2B:16905748..16907712 | Protein kinase, putative |
| TraesCS2B01G035100.1 | chr2B:16958038..16960745 | Disease resistance protein RPM1 |
| TraesCS2B01G035200.1 | chr2B:16968939..16970962 | Glutamyl-tRNA (Gln) amidotransferase subunit A |
| TraesCS2B01G035300.1 | chr2B:16988998..16991703 | receptor kinase 1 |
| TraesCS2B01G035400.1 | chr2B:17023215..17026818 | receptor kinase 1 |
| TraesCS2B01G035500.1 | chr2B:17039784..17041867 | Protein UPSTREAM OF FLC |
| TraesCS2B01G035500.2 | chr2B:17039816..17041723 | Protein UPSTREAM OF FLC |

| | | |
|---|---|---|
| TraesCS2B01G035600.1 | chr2B:17109017..17112288 | Cytochrome P450 |
| TraesCS2B01G035700.1 | chr2B:17144025..17150502 | Cytochrome P450 |
| TraesCS2B01G035800.1 | chr2B:17153049..17161879 | Glutamyl-tRNA (Gln) amidotransferase subunit A |
| TraesCS2B01G035900.1 | chr2B:17162074..17164381 | Glutamyl-tRNA (Gln) amidotransferase subunit A |
| TraesCS2B01G036000.1 | chr2B:17170842..17172366 | Glutamyl-tRNA (Gln) amidotransferase subunit A |
| TraesCS2B01G036100.1 | chr2B:17202140..17203059 | Glutamyl-tRNA (Gln) amidotransferase subunit A |
| TraesCS2B01G036200.1 | chr2B:17235281..17240043 | Receptor kinase-like protein |
| TraesCS2B01G036300.1 | chr2B:17342933..17346233 | Carboxyl methyltransferase |
| TraesCS2B01G036400.1 | chr2B:17364135..17367551 | Receptor-like protein kinase |
| TraesCS2B01G036500.1 | chr2B:17367858..17371851 | Vesicle-associated 1-1-like protein |
| TraesCS2B01G036600.1 | chr2B:17388763..17391444 | receptor lectin kinase |
| TraesCS2B01G036700.1 | chr2B:17394003..17400286 | receptor kinase 1 |
| TraesCS2B01G036700.2 | chr2B:17394003..17400286 | receptor kinase 1 |
| TraesCS2B01G036800.1 | chr2B:17430640..17433898 | receptor kinase 1 |
| TraesCS2B01G036900.1 | chr2B:17436964..17440247 | Cytochrome P450 |
| TraesCS2B01G037000.1 | chr2B:17444363..17445584 | Cytochrome P450 |
| TraesCS2B01G037100.1 | chr2B:17447553..17450991 | Glutamyl-tRNA (Gln) amidotransferase subunit A |
| TraesCS2B01G037200.1 | chr2B:17452555..17461151 | Glutamyl-tRNA (Gln) amidotransferase subunit A |
| TraesCS2B01G037300.1 | chr2B:17464732..17466728 | Receptor kinase-like protein |
| TraesCS2B01G037400.1 | chr2B:17485319..17492068 | Glutamyl-tRNA (Gln) amidotransferase subunit A |
| TraesCS2B01G037500.1 | chr2B:17494934..17498341 | Glutamyl-tRNA (Gln) amidotransferase subunit A |
| TraesCS2B01G037600.1 | chr2B:17501517..17503262 | Cytochrome P450 |
| TraesCS2B01G037700.1 | chr2B:17513541..17515328 | Cytochrome P450 |
| TraesCS2B01G037800.1 | chr2B:17518406..17521533 | Glutamyl-tRNA (Gln) amidotransferase subunit A |
| TraesCS2B01G037900.1 | chr2B:17564240..17573639 | Disease resistance protein (NBS-LRR class) family |

| | | |
|---|---|---|
| TraesCS2B01G038000.1 | chr2B:17593209..17599029 | receptor kinase 1 |
| TraesCS2B01G038100.1 | chr2B:17697606..17704187 | NBS-LRR-like resistance protein |
| TraesCS2B01G038100.2 | chr2B:17697606..17704187 | NBS-LRR-like resistance protein |
| TraesCS2B01G038200.1 | chr2B:17778903..17795505 | Glycine-rich protein |
| TraesCS2B01G038300.1 | chr2B:17824092..17827254 | Subtilisin-like protease |
| TraesCS2B01G038400.1 | chr2B:17827297..17831181 | Receptor protein kinase, putative |
| TraesCS2B01G038500.1 | chr2B:17835567..17838278 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein |
| TraesCS2B01G038600.1 | chr2B:17872742..17874073 | Chalcone synthase |
| TraesCS2B01G038700.1 | chr2B:17881615..17883403 | Chalcone synthase |
| TraesCS2B01G038800.1 | chr2B:17925622..17930956 | NBS-LRR-like resistance protein |
| TraesCS2B01G038900.1 | chr2B:17933889..17939476 | Disease resistance protein (NBS-LRR class) family |
| TraesCS2B01G039000.1 | chr2B:17994890..17995279 | DNA topoisomerase 3-alpha |
| TraesCS2B01G039100.1 | chr2B:18010522..18018422 | NBS-LRR-like resistance protein |
| TraesCS2B01G039200.1 | chr2B:18067815..18074650 | Disease resistance protein RPM1 |
| TraesCS2B01G039300.1 | chr2B:18090261..18094555 | Disease resistance protein RPP13 |
| TraesCS2B01G039400.1 | chr2B:18092234..18094555 | Disease resistance protein (TIR-NBS-LRR class) family |
| TraesCS2B01G039500.1 | chr2B:18108608..18114962 | Disease resistance protein (NBS-LRR class) family |
| TraesCS2B01G039600.1 | chr2B:18122376..18128032 | Disease resistance protein (NBS-LRR class) family |
| TraesCS2B01G039700.1 | chr2B:18136604..18142875 | Disease resistance protein RPP13 |
| TraesCS2B01G039800.1 | chr2B:18164295..18171733 | Disease resistance protein RPM1 |

| | | |
|---|---|---|
| TraesCS2B01G039900.1 | chr2B:18174875..18177112 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein, putative |
| TraesCS2B01G039900.2 | chr2B:18174875..18177112 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein, putative |
| TraesCS2B01G040000.1 | chr2B:18177103..18178744 | 12-oxophytodienoate reductase-like protein |
| TraesCS2B01G040100.1 | chr2B:18196456..18198001 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein, putative |
| TraesCS2B01G040200.1 | chr2B:18260602..18263620 | HR-like lesion-inducing protein-related protein |
| TraesCS2B01G040300.1 | chr2B:18264092..18276240 | Actin-related protein |
| TraesCS2B01G040400.1 | chr2B:18306127..18311626 | Copalyl diphosphate synthase |
| TraesCS2B01G040500.1 | chr2B:18319166..18320973 | Glycosyltransferase |
| TraesCS2B01G040600.1 | chr2B:18385568..18387362 | Glycosyltransferase |
| TraesCS2B01G040700.1 | chr2B:18658522..18664477 | Agenet domain, putative |
| TraesCS2B01G040800.1 | chr2B:18682032..18704233 | Copalyl diphosphate synthase |
| TraesCS2B01G040900.1 | chr2B:18710118..18715097 | Kaurene synthase |
| TraesCS6A01G365500.1 | chr6A:593732776..593733555 | Serine-rich protein |
| TraesCS6A01G365600.1 | chr6A:593774891..593775421 | Chromosome partition protein MukB |
| TraesCS6A01G365700.1 | chr6A:593777061..593777987 | Serine-rich protein |
| TraesCS6A01G365800.1 | chr6A:593785652..593786529 | Serine-rich protein |
| TraesCS6A01G365900.1 | chr6A:593792241..593792983 | Serine-rich protein |
| TraesCS6A01G366000.1 | chr6A:593807939..593809154 | Serine-rich protein |
| TraesCS6A01G366100.1 | chr6A:593928982..593929970 | Serine-rich protein |
| TraesCS6A01G366200.1 | chr6A:593991705..593992418 | Serine-rich protein |
| TraesCS6A01G366300.1 | chr6A:594073722..594074596 | Werner Syndrome-like exonuclease |

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|---|---|---|
| TraesCS6A01G366400.1 | chr6A:594253281..594254269 | Serine-rich protein |
| TraesCS6A01G366500.1 | chr6A:594253682..594254125 | maternal effect embryo arrest 22 |
| TraesCS6A01G366600.1 | chr6A:594279366..594279620 | Unknown protein |
| TraesCS6A01G366700.1 | chr6A:594319219..594320257 | Serine-rich protein |
| TraesCS6A01G366800.1 | chr6A:594365660..594366568 | Serine-rich protein |
| TraesCS6A01G366900.1 | chr6A:594367508..594368349 | Cupin, RmlC-type |
| TraesCS6A01G367000.1 | chr6A:594405188..594408225 | Bifunctional nuclease |
| TraesCS6A01G367000.2 | chr6A:594405188..594408225 | Bifunctional nuclease |
| TraesCS6A01G367100.1 | chr6A:594430046..594435343 | CobW-domain-containing protein |
| TraesCS6A01G367200.1 | chr6A:594440042..594443601 | Proteasome maturation factor UMP1 family protein |
| TraesCS6A01G367300.1 | chr6A:594492084..594496424 | Heparanase |
| TraesCS6A01G367300.2 | chr6A:594492084..594496451 | Heparanase |
| TraesCS6A01G367400.1 | chr6A:594497828..594499003 | BTB/POZ domain containing protein |
| TraesCS6A01G367500.1 | chr6A:594502555..594503772 | Prostamide/prostaglandin F synthase |
| TraesCS6A01G367600.1 | chr6A:594504073..594506121 | F-box and associated interaction domains-containing protein |
| TraesCS6A01G367700.1 | chr6A:594515148..594516412 | BTB/POZ domain containing protein, expressed |
| TraesCS6A01G367800.1 | chr6A:594519604..594521270 | Leucine-rich repeat protein kinase family protein |
| TraesCS6A01G367900.1 | chr6A:594523338..594530536 | BTB/POZ domain containing protein |
| TraesCS6A01G368000.1 | chr6A:594531841..594537909 | Calcineurin B-like protein |
| TraesCS6A01G368100.1 | chr6A:594531841..594537909 | Glutamate receptor |
| TraesCS6A01G368200.1 | chr6A:594608390..594610244 | Peroxidase |
| TraesCS6A01G368300.1 | chr6A:594641968..594644848 | E3 ubiquitin-protein ligase SINA-like 2 |
| TraesCS6A01G368400.1 | chr6A:594652219..594654001 | Peroxidase |
| TraesCS6A01G368500.1 | chr6A:594661270..594662474 | Sulfotransferase |

| | | |
|---|---|---|
| TraesCS6A01G368600.1 | chr6A:594728835..594733356 | RNA binding |
| TraesCS6A01G368700.1 | chr6A:594747830..594753642 | UDP-N-acetylglucosamine--N-acetylmuramyl-(Pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase |
| TraesCS6A01G368800.1 | chr6A:594754235..594755829 | Mitochondrial group I intron splicing factor CCM1 |
| TraesCS6A01G368900.1 | chr6A:594819237..594822022 | 30S ribosomal protein S15 |
| TraesCS6A01G369000.1 | chr6A:594823779..594834502 | N-alpha-acetyltransferase 35, NatC auxiliary subunit |
| TraesCS6A01G369000.2 | chr6A:594823779..594834646 | N-alpha-acetyltransferase 35, NatC auxiliary subunit |
| TraesCS6A01G369000.3 | chr6A:594823779..594834646 | N-alpha-acetyltransferase 35, NatC auxiliary subunit |
| TraesCS6A01G369100.1 | chr6A:594854000..594857470 | F-box family protein |
| TraesCS6A01G369100.2 | chr6A:594854038..594857358 | F-box family protein |
| TraesCS6A01G369200.1 | chr6A:594947384..594951245 | F-box family protein |
| TraesCS6A01G369300.1 | chr6A:594958023..594959979 | GDSL esterase/lipase |
| TraesCS6A01G369400.1 | chr6A:594966063..594979269 | F-box family protein |
| TraesCS6A01G369500.1 | chr6A:594979212..594983369 | Rhomboid family protein |
| TraesCS6A01G369600.1 | chr6A:594985124..594990860 | F-box family protein |
| TraesCS6A01G369700.1 | chr6A:595002723..595004774 | Flavonoid 3'-hydroxylase |
| TraesCS6A01G369800.1 | chr6A:595023602..595029693 | Alcohol dehydrogenase, putative |
| TraesCS6A01G369900.1 | chr6A:595058778..595061633 | Peptide transporter |
| TraesCS6A01G370000.1 | chr6A:595065568..595066265 | F-box family protein |
| TraesCS6A01G370100.1 | chr6A:595070384..595077134 | Alcohol dehydrogenase, putative |
| TraesCS6A01G370200.1 | chr6A:595177921..595181621 | F-box family protein |
| TraesCS6A01G370300.1 | chr6A:595182781..595186595 | Acetamidase/formamidase, putative |
| TraesCS6A01G370400.1 | chr6A:595328220..595330827 | F-box family protein |

| | | |
|---|---|---|
| TraesCS6A01G370500.1 | chr6A:595475968..595480317 | ENTH/ANTH/VHS superfamily protein |
| TraesCS6A01G370600.1 | chr6A:595480643..595482789 | Vacuolar protein sorting 55 containing protein |
| TraesCS6A01G370700.1 | chr6A:595487025..595490733 | F-box family protein |
| TraesCS6A01G370700.2 | chr6A:595487025..595491526 | F-box family protein |
| TraesCS6A01G370800.1 | chr6A:595498114..595501654 | Aspartic proteinase nepenthesin-1 |
| TraesCS6A01G370900.1 | chr6A:595507215..595510673 | Powder tolerance-related protein |
| TraesCS6A01G371000.1 | chr6A:595563491..595564356 | Defensin-like (DEFL) family protein |
| TraesCS6A01G371100.1 | chr6A:595608368..595614056 | Aldehyde dehydrogenase |
| TraesCS6A01G371200.1 | chr6A:595621908..595624661 | Pentatricopeptide repeat-containing protein |
| TraesCS6A01G371300.1 | chr6A:595624898..595628331 | oxidoreductase/transition metal ion-binding protein (DUF3531) |
| TraesCS6A01G371400.1 | chr6A:595648138..595659525 | Little zipper protein |
| TraesCS6A01G371500.1 | chr6A:595648138..595659525 | Little zipper protein |
| TraesCS6A01G371600.1 | chr6A:595662087..595665070 | F-box family protein |
| TraesCS6A01G371700.1 | chr6A:595670870..595677042 | Two-component response regulator |
| TraesCS6A01G371800.1 | chr6A:595774891..595778255 | Vesicle-associated membrane protein |
| TraesCS7A01G529200.1 | chr7A:709129307..709142747 | Chaperone protein dnaJ |
| TraesCS7A01G529300.1 | chr7A:709164507..709169937 | Nucleobase ascorbate transporter |
| TraesCS7A01G529400.1 | chr7A:709175588..709176337 | Chaperone protein dnaJ |
| TraesCS7A01G529500.1 | chr7A:709261362..709263804 | Pathogenesis-related thaumatin family protein |
| TraesCS7A01G529600.1 | chr7A:709263863..709266331 | F-box domain containing protein |
| TraesCS7A01G529700.1 | chr7A:709263863..709266331 | F-box family protein |
| TraesCS7A01G529700.2 | chr7A:709263863..709266331 | F-box family protein |

| | | |
|---|---|---|
| TraesCS7A01G529800.1 | chr7A:709274149..709274700 | Methylthioribose-1-phosphate isomerase |
| TraesCS7A01G529900.1 | chr7A:709404520..709410244 | Chaperone protein htpG family protein |
| TraesCS7A01G529900.2 | chr7A:709404520..709410244 | Chaperone protein htpG family protein |
| TraesCS7A01G530000.1 | chr7A:709424535..709424926 | Eukaryotic translation initiation factor 3 subunit B |
| TraesCS7A01G530100.1 | chr7A:709549290..709552176 | transmembrane protein, putative (DUF594) |
| TraesCS7A01G530200.1 | chr7A:709586964..709589986 | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein |
| TraesCS7A01G530300.1 | chr7A:709638392..709643676 | Transcription factor |
| TraesCS7A01G530400.1 | chr7A:709674288..709675195 | BZIP transcription factor |
| TraesCS7A01G530500.1 | chr7A:709677708..709679112 | F-box family protein, putative |
| TraesCS7A01G530600.1 | chr7A:709697901..709699391 | F-box family protein, putative |
| TraesCS7A01G530700.1 | chr7A:709736367..709739530 | Pentatricopeptide repeat-containing protein |
| TraesCS7A01G530800.1 | chr7A:709784096..709785694 | Cysteine/histidine-rich C1 domain-containing protein |
| TraesCS7A01G530900.1 | chr7A:709790183..709794933 | LINE-1 reverse transcriptase-like protein |
| TraesCS7A01G531000.1 | chr7A:709808961..709813538 | Peptide transporter |
| TraesCS7A01G531000.2 | chr7A:709808961..709813538 | Peptide transporter |
| TraesCS7A01G531100.1 | chr7A:709824905..709825969 | Glutathione S-transferase |
| TraesCS7A01G531200.1 | chr7A:709853862..709863775 | Disease resistance protein RPM1 |
| TraesCS7A01G531300.1 | chr7A:709870813..709872600 | LEUNIG-like protein |
| TraesCS7A01G531400.1 | chr7A:709888762..709893282 | Disease resistance protein RPM1 |
| TraesCS7A01G531500.1 | chr7A:709965453..709977412 | Cytosine-specific methyltransferase |

| | | |
|---|---|---|
| TraesCS7A01G531600.1 | chr7A:709985456..709985752 | Polynucleotidyl transferase, Ribonuclease H fold |
| TraesCS7A01G531700.1 | chr7A:709984066..709990726 | F-box protein PP2 |
| TraesCS7A01G531800.1 | chr7A:709998043..709998978 | Basic-leucine zipper (bZIP) transcription factor family protein |
| TraesCS7A01G531900.1 | chr7A:710502090..710503493 | BTB/POZ domain containing protein |
| TraesCS7A01G532000.1 | chr7A:710832209..710836948 | Protein phosphatase 2C-like protein |
| TraesCS7A01G532100.1 | chr7A:710957563..710959194 | 3-ketoacyl-CoA synthase |
| TraesCS7A01G532200.1 | chr7A:710957563..710959194 | 3-ketoacyl-CoA synthase |
| TraesCS7A01G532300.1 | chr7A:711170844..711172723 | 3-ketoacyl-CoA synthase |
| TraesCS7B01G472000.1 | chr7B:728005673..728006521 | Zinc finger family protein |
| TraesCS7B01G472100.1 | chr7B:728012723..728013631 | Zinc finger family protein |
| TraesCS7B01G472200.1 | chr7B:728287636..728288820 | NAC domain-containing protein |
| TraesCS7B01G472300.1 | chr7B:728624206..728630807 | 1,4-alpha-glucan branching enzyme |
| TraesCS7B01G472300.2 | chr7B:728624215..728630807 | 1,4-alpha-glucan branching enzyme |
| TraesCS7B01G472400.1 | chr7B:728665334..728684619 | 1,4-alpha-glucan branching enzyme |
| TraesCS7B01G472500.1 | chr7B:728689640..728696485 | 1,4-alpha-glucan branching enzyme |
| TraesCS7B01G472500.2 | chr7B:728689640..728696485 | 1,4-alpha-glucan branching enzyme |
| TraesCS7B01G472600.1 | chr7B:728699511..728700949 | NAC domain-containing protein |
| TraesCS7B01G472700.1 | chr7B:728807189..728812239 | Transmembrane protein 184C |
| TraesCS7B01G472800.1 | chr7B:728834600..728838454 | Cyclin family protein |
| TraesCS7B01G472900.1 | chr7B:728933649..728935903 | Cytochrome P450, putative |
| TraesCS7B01G473000.1 | chr7B:728964055..728966226 | F-box protein skip23 |

| | | |
|---|---|---|
| TraesCS7B01G473100.1 | chr7B:729024348..729024685 | Polynucleotidyl transferase, ribonuclease H-like superfamily protein |
| TraesCS7B01G473200.1 | chr7B:730022756..730025414 | U-box domain-containing protein |

**Appendix 7.1** Results of the putative candidate genes associated with BYD resistance on chromosome 5A.

| Annotated gene ID | Physical position | Putative function |
|---|---|---|
| TraesCS5A01G431400.1 | chr5A:615301432..615307285 | F-box protein |
| TraesCS5A01G431500.1 | chr5A:615307659..615310876 | F-box/FBD/LRR-repeat protein |
| TraesCS5A01G431600.1 | chr5A:615312042..615319080 | Kinase family protein |
| TraesCS5A01G431700.1 | chr5A:615862325..615864769 | F-box family protein |
| TraesCS5A01G431700.2 | chr5A:615862325..615864769 | F-box family protein |
| TraesCS5A01G431800.1 | chr5A:616064265..616070267 | F-box domain containing protein-like |
| TraesCS5A01G431900.1 | chr5A:616145787..616149329 | Protein translocase subunit SecA 2 |
| TraesCS5A01G432000.1 | chr5A:616517967..616523558 | Type I inositol-1,4,5-trisphosphate 5-phosphatase CVP2 |
| TraesCS5A01G432100.1 | chr5A:616547318..616549433 | Globulin-1 |
| TraesCS5A01G432200.1 | chr5A:616621360..616622374 | Proteasome subunit beta type |
| TraesCS5A01G432300.1 | chr5A:616648688..616652543 | Ripening related protein family |
| TraesCS5A01G432400.1 | chr5A:616805000..616805779 | Ripening related protein family |
| TraesCS5A01G432500.1 | chr5A:616855369..616860167 | Ripening related protein family |
| TraesCS5A01G432600.1 | chr5A:616878504..616879731 | Glutathione S-transferase |
| TraesCS5A01G432700.1 | chr5A:616908983..616909930 | Lipid transfer protein |
| TraesCS5A01G432800.1 | chr5A:616913769..616914660 | Lipid transfer protein |
| TraesCS5A01G432900.1 | chr5A:616919188..616920006 | Lipid transfer protein |
| TraesCS5A01G433000.1 | chr5A:616978423..616979255 | Lipid transfer protein |
| TraesCS5A01G433100.1 | chr5A:616981515..616982613 | Non-specific lipid-transfer protein-like protein |
| TraesCS5A01G433200.1 | chr5A:617095685..617097491 | Glycosyltransferase |
| TraesCS5A01G433300.1 | chr5A:617121840..617123786 | Glycosyltransferase |
| TraesCS5A01G433400.1 | chr5A:617173503..617174817 | Lipid transfer protein |
| TraesCS5A01G433500.1 | chr5A:617174968..617176025 | Lipid transfer protein |

| | | |
|---|---|---|
| TraesCS5A01G433600.1 | chr5A:617182738..617186631 | receptor kinase 1 |
| TraesCS5A01G433600.2 | chr5A:617182738..617186631 | receptor kinase 1 |
| TraesCS5A01G433700.1 | chr5A:617187408..617188186 | Acetyltransferase (GNAT) domain protein |
| TraesCS5A01G433800.1 | chr5A:617188181..617189235 | Lipid transfer protein |
| TraesCS5A01G433900.1 | chr5A:617204247..617205195 | Acetyltransferase, GNAT family protein, expressed |
| TraesCS5A01G434000.1 | chr5A:617205877..617208108 | Acetyltransferase, GNAT family protein, expressed |
| TraesCS5A01G434100.1 | chr5A:617207272..617212776 | Glutamate dehydrogenase |
| TraesCS5A01G434200.1 | chr5A:617235790..617237619 | 30S ribosomal protein S13 |
| TraesCS5A01G434300.1 | chr5A:617251251..617255351 | Serine/threonine-protein kinase |
| TraesCS5A01G434400.1 | chr5A:617346820..617357344 | 5'-3' exoribonuclease, putative, expressed |
| TraesCS5A01G434400.2 | chr5A:617346820..617357344 | 5'-3' exoribonuclease, putative, expressed |
| TraesCS5A01G434500.1 | chr5A:617357806..617361664 | Protein FRIGIDA |
| TraesCS5A01G434500.2 | chr5A:617357806..617361664 | Protein FRIGIDA |
| TraesCS5A01G434600.1 | chr5A:617365771..617367566 | Zinc finger protein, putative |
| TraesCS5A01G434700.1 | chr5A:617953834..617955478 | Pectinesterase |
| TraesCS5A01G434800.1 | chr5A:618126122..618127107 | BTB/POZ domain containing protein |
| TraesCS5A01G434900.1 | chr5A:618158444..618160012 | BTB/POZ domain containing protein |
| TraesCS5A01G435000.1 | chr5A:618270410..618271759 | BTB/POZ domain containing protein |
| TraesCS5A01G435100.1 | chr5A:618307187..618308493 | BTB/POZ domain containing protein, expressed |
| TraesCS5A01G435200.1 | chr5A:618477854..618479282 | BTB/POZ domain containing protein, expressed |

| | | |
|---|---|---|
| TraesCS5A01G435300.1 | chr5A:618602471..618604001 | BTB/POZ domain containing protein, expressed |
| TraesCS5A01G435400.1 | chr5A:618665608..618667352 | GDSL esterase/lipase |
| TraesCS5A01G435500.1 | chr5A:618944372..618945980 | E3 ubiquitin-protein ligase |
| TraesCS5A01G435600.1 | chr5A:618949672..618954260 | Folate/biopterin transporter family protein, expressed |
| TraesCS5A01G435600.2 | chr5A:618949067..618954260 | Folate/biopterin transporter family protein, expressed |
| TraesCS5A01G435700.1 | chr5A:618960407..618964664 | Queuine tRNA-ribosyltransferase, putative |
| TraesCS5A01G435800.1 | chr5A:618965319..618968709 | Low affinity potassium transport system protein |
| TraesCS5A01G435800.2 | chr5A:618965319..618968709 | Disease resistance protein (CC-NBS-LRR class) family |
| TraesCS5A01G435900.1 | chr5A:619091714..619093991 | Pentatricopeptide repeat-containing protein |
| TraesCS5A01G435900.2 | chr5A:619091714..619094213 | Pentatricopeptide repeat-containing protein |
| TraesCS5A01G436000.1 | chr5A:619097699..619099222 | Leucine-rich repeat protein kinase family protein |
| TraesCS5A01G436100.1 | chr5A:619106645..619109698 | Pentatricopeptide repeat-containing protein |
| TraesCS5A01G436200.1 | chr5A:619109755..619115181 | Ankyrin repeat family protein, putative, expressed |
| TraesCS5A01G436300.1 | chr5A:619172748..619179227 | Methionine S-methyltransferase |
| TraesCS5A01G436300.2 | chr5A:619172748..619181657 | Methionine S-methyltransferase |
| TraesCS5A01G436400.1 | chr5A:619255848..619266482 | 50S ribosomal protein L22 |
| TraesCS5A01G436500.1 | chr5A:619264485..619266482 | 30S ribosomal protein S19, chloroplastic |
| TraesCS5A01G436600.1 | chr5A:619264497..619266057 | Potassium-transporting ATPase potassium-binding subunit |

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|---|---|---|
| TraesCS5A01G436700.1 | chr5A:619264485..619266482 | 50S ribosomal protein L2 |
| TraesCS5A01G436800.1 | chr5A:619264497..619267223 | Ribosomal protein L2 |
| TraesCS5A01G436900.1 | chr5A:619267030..619267298 | 50S ribosomal protein L23 |
| TraesCS5A01G437000.1 | chr5A:619273593..619273736 | Photosystem II reaction center protein J |
| TraesCS5A01G437100.1 | chr5A:619273840..619273956 | Photosystem II reaction center protein L |
| TraesCS5A01G437200.1 | chr5A:619359773..619361549 | Glycosyltransferase |
| TraesCS5A01G437300.1 | chr5A:619454129..619455767 | Alpha/beta-Hydrolases superfamily protein, putative |
| TraesCS5A01G437400.1 | chr5A:619467274..619468718 | Transcription initiation factor IIA subunit 1 |
| TraesCS5A01G437500.1 | chr5A:619470624..619473688 | Transcription initiation factor IIA subunit 1 |
| TraesCS5A01G437600.1 | chr5A:619679147..619682531 | PITH domain-containing protein |
| TraesCS5A01G437700.1 | chr5A:619682572..619683616 | Bax inhibitor-1 family protein |
| TraesCS5A01G437800.1 | chr5A:619684658..619688576 | Bax inhibitor-1 family protein |
| TraesCS5A01G437900.1 | chr5A:619689395..619693287 | Heat shock transcription factor |
| TraesCS5A01G437900.2 | chr5A:619689395..619693287 | Heat shock transcription factor |
| TraesCS5A01G437900.3 | chr5A:619689395..619693287 | Heat shock transcription factor |
| TraesCS5A01G438000.1 | chr5A:619693302..619694794 | Transmembrane protein 97, Putative |
| TraesCS5A01G438100.1 | chr5A:619714609..619718660 | TPR domain containing protein |
| TraesCS5A01G438200.1 | chr5A:619724172..619725749 | B3 domain protein (DUF313) |
| TraesCS5A01G438300.1 | chr5A:619753481..619754737 | Transmembrane protein 97 |

| | | |
|---|---|---|
| TraesCS5A01G438400.1 | chr5A:619773727..619774891 | Transposon protein, putative, CACTA, En/Spm sub-class |
| TraesCS5A01G438500.1 | chr5A:619852506..619853880 | B3 domain-containing protein |
| TraesCS5A01G438600.1 | chr5A:619955073..619959823 | B3 domain-containing protein |
| TraesCS5A01G438700.1 | chr5A:619966103..619970761 | B3 domain-containing protein |
| TraesCS5A01G438800.1 | chr5A:620175114..620179180 | Stem-specific protein TSJT1 |
| TraesCS5A01G438900.1 | chr5A:620400166..620401190 | CASP-like protein |
| TraesCS5A01G439000.1 | chr5A:620478851..620481470 | Glyceraldehyde-3-phosphate dehydrogenase 2 |
| TraesCS5A01G439100.1 | chr5A:620560637..620563210 | 60S ribosomal protein L4 |
| TraesCS5A01G439100.2 | chr5A:620560637..620563210 | 60S ribosomal protein L4 |
| TraesCS5A01G439200.1 | chr5A:620856674..620859198 | DUF506 family protein |
| TraesCS5A01G439300.1 | chr5A:620993717..620996088 | U3 small nucleolar RNA-associated protein 18-like protein |
| TraesCS5A01G439400.1 | chr5A:620996257..621003483 | Protein ENHANCED DOWNY MILDEW 2 |
| TraesCS5A01G439400.2 | chr5A:620996257..621003483 | Histone-lysine N-methyltransferase NSD3 |
| TraesCS5A01G439500.1 | chr5A:621006636..621006971 | Nuclear receptor subfamily 2 group C member 1 |
| TraesCS5A01G439600.1 | chr5A:621009037..621010261 | U3 small nucleolar RNA-associated protein 18-like protein |
| TraesCS5A01G439700.1 | chr5A:621023309..621024094 | Pathogenesis-related protein 1 |
| TraesCS5A01G439800.1 | chr5A:621177046..621177918 | Pathogenesis-related protein 1 |
| TraesCS5A01G439900.1 | chr5A:621444194..621449202 | Pathogenesis-related protein 1 |

| | | |
|---|---|---|
| TraesCS5A01G440000.1 | chr5A:621528221..621529025 | Pathogenesis-related protein 1 |
| TraesCS5A01G440100.1 | chr5A:621538571..621540609 | Mannitol transporter, putative, expressed |
| TraesCS5A01G440200.1 | chr5A:621745275..621745741 | myb domain protein 65 |
| TraesCS5A01G440300.1 | chr5A:621746705..621759116 | Guanylate-binding family protein, putative, expressed |
| TraesCS5A01G440400.1 | chr5A:621759394..621764201 | Basic-leucine zipper (BZIP) transcription factor family |
| TraesCS5A01G440400.2 | chr5A:621759394..621764201 | Basic-leucine zipper (BZIP) transcription factor family |
| TraesCS5A01G440500.1 | chr5A:621845909..621849870 | BTB/POZ domain containing protein |
| TraesCS5A01G440600.1 | chr5A:621850747..621855278 | Disease resistance protein (NBS-LRR class) family |
| TraesCS5A01G440700.1 | chr5A:621855523..621860591 | Tryptophan synthase alpha chain |
| TraesCS5A01G440800.1 | chr5A:621861000..621867855 | Tryptophan synthase alpha chain |
| TraesCS5A01G440900.1 | chr5A:621882228..621884505 | Tryptophan synthase alpha chain |
| TraesCS5A01G441000.1 | chr5A:622046270..622053415 | Pseudouridine synthase |
| TraesCS5A01G441100.1 | chr5A:622192881..622195617 | Tryptophan synthase alpha chain |
| TraesCS5A01G441100.2 | chr5A:622192881..622195617 | Tryptophan synthase alpha chain |
| TraesCS5A01G441200.1 | chr5A:622200986..622207580 | ARM repeat superfamily protein |
| TraesCS5A01G441300.1 | chr5A:622224571..622226834 | Tryptophan synthase alpha chain |
| TraesCS5A01G441400.1 | chr5A:622232361..622234610 | Endo-1,4-beta-xylanase |
| TraesCS5A01G441500.1 | chr5A:622296130..622299544 | p-loop containing nucleoside triphosphate |

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|---|---|---|
| | | hydrolases superfamily protein, putative |
| TraesCS5A01G441600.1 | chr5A:622392176..622394450 | Endo-1,4-beta-xylanase |
| TraesCS5A01G441700.1 | chr5A:622458677..622460866 | Endo-1,4-beta-xylanase |
| TraesCS5A01G441800.1 | chr5A:622540668..622542039 | F-box protein |
| TraesCS5A01G441900.1 | chr5A:622543627..622548765 | Basic helix-loop-helix transcription factor |
| TraesCS5A01G442000.1 | chr5A:622551327..622556320 | rRNA-processing protein efg1 |
| TraesCS5A01G442000.2 | chr5A:622551327..622556320 | rRNA-processing protein efg1 |
| TraesCS5A01G442100.1 | chr5A:622764667..622766144 | Leucine-rich repeat receptor-like protein kinase family protein |
| TraesCS5A01G442200.1 | chr5A:622767734..622773588 | Lysine--tRNA ligase |
| TraesCS5A01G442200.2 | chr5A:622767734..622773588 | Lysine--tRNA ligase |
| TraesCS5A01G442300.1 | chr5A:622773720..622780594 | DNA-binding protein SMUBP-2 |
| TraesCS5A01G442400.1 | chr5A:622780537..622783379 | Purple acid phosphatase |
| TraesCS5A01G442500.1 | chr5A:622986145..622990813 | Eukaryotic translation initiation factor 3 subunit B |
| TraesCS5A01G442600.1 | chr5A:622991620..622992484 | Pre-mRNA-splicing factor 3 |
| TraesCS5A01G442700.1 | chr5A:622997700..623004364 | Ankyrin repeat protein-like |
| TraesCS5A01G442800.1 | chr5A:623021953..623026624 | Diphosphomevalonate decarboxylase |
| TraesCS5A01G442900.1 | chr5A:623205806..623207360 | F-box protein, putative (DUF295) |
| TraesCS5A01G443000.1 | chr5A:623207461..623213608 | E3 ubiquitin protein ligase DRIP2 |
| TraesCS5A01G443000.2 | chr5A:623207461..623213608 | E3 ubiquitin protein ligase DRIP2 |
| TraesCS5A01G443000.3 | chr5A:623207461..623213608 | E3 ubiquitin protein ligase DRIP2 |
| TraesCS5A01G443100.1 | chr5A:623214050..623215943 | Trihelix transcription factor |

| | | |
|---|---|---|
| TraesCS5A01G443200.1 | chr5A:623487134..623492214 | Cytosine-specific methyltransferase |
| TraesCS5A01G443300.1 | chr5A:623492594..623493856 | PRA1 family protein |
| TraesCS5A01G443400.1 | chr5A:623503258..623504630 | Expansin |
| TraesCS5A01G443500.1 | chr5A:623552324..623556496 | Tubulin beta chain |
| TraesCS5A01G443600.1 | chr5A:623565130..623567588 | WRKY transcription factor, putative |
| TraesCS5A01G443700.1 | chr5A:623571600..623574243 | 30S ribosomal protein S19 |
| TraesCS5A01G443800.1 | chr5A:623685045..623686449 | Histone H1 |
| TraesCS5A01G443900.1 | chr5A:623954938..623956160 | Histone H1 |
| TraesCS5A01G443900.2 | chr5A:623954938..623956160 | Histone H1 |
| TraesCS5A01G444000.1 | chr5A:623957098..623957508 | cytochrome P450, family 705, subfamily A, polypeptide 15 |
| TraesCS5A01G444100.1 | chr5A:624209630..624212676 | myosin-binding protein (Protein of unknown function, DUF593) |
| TraesCS5A01G444200.1 | chr5A:624213254..624218588 | TPX2 (Targeting protein for Xklp2) family protein |
| TraesCS5A01G444300.1 | chr5A:624473907..624475399 | Histone H1 |
| TraesCS5A01G444400.1 | chr5A:624677592..624681176 | Coiled-coil alpha-helical rod protein 1 |
| TraesCS5A01G444500.1 | chr5A:624706397..624708821 | Glycosyltransferase |
| TraesCS5A01G444600.1 | chr5A:624710507..624714881 | Receptor-like protein kinase |
| TraesCS5A01G444700.1 | chr5A:624721961..624726908 | Receptor-like protein kinase |
| TraesCS5A01G444800.1 | chr5A:624733672..624737958 | carboxyl-terminal peptidase (DUF239) |
| TraesCS5A01G444900.1 | chr5A:624739259..624744656 | receptor kinase 1 |
| TraesCS5A01G445000.1 | chr5A:624744063..624760470 | Jacalin-related lectin |
| TraesCS5A01G445000.2 | chr5A:624744063..624760470 | Jacalin-related lectin |
| TraesCS5A01G445100.1 | chr5A:624787241..624792887 | GTPase obg |
| TraesCS5A01G445200.1 | chr5A:624793113..624797087 | BAH-PHD domain-containing protein |

| | | |
|---|---|---|
| TraesCS5A01G445200.2 | chr5A:624793113..624797087 | BAH-PHD domain-containing protein |
| TraesCS5A01G445200.3 | chr5A:624793113..624797087 | BAH-PHD domain-containing protein |
| TraesCS5A01G445300.1 | chr5A:624799322..624801780 | Protein REVERSION-TO-ETHYLENE SENSITIVITY1 |
| TraesCS5A01G445400.1 | chr5A:624965124..624967466 | Polyubiquitin |
| TraesCS5A01G445500.1 | chr5A:625419723..625420214 | Ycf68 |
| TraesCS5A01G445600.1 | chr5A:625715327..625716159 | Histone H2A |
| TraesCS5A01G445700.1 | chr5A:625732302..625742082 | Receptor-like protein kinase |
| TraesCS5A01G445800.1 | chr5A:625908227..625909500 | Auxin efflux carrier family protein |
| TraesCS5A01G445900.1 | chr5A:626024140..626030662 | Small multi-drug export protein |
| TraesCS5A01G446000.1 | chr5A:626059348..626067611 | Argonaute protein |
| TraesCS5A01G446000.2 | chr5A:626061264..626067611 | Argonaute protein |
| TraesCS5A01G446100.1 | chr5A:626068558..626076649 | Cactin |
| TraesCS5A01G446200.1 | chr5A:626084072..626089054 | OTU domain-containing protein |
| TraesCS5A01G446300.1 | chr5A:626110879..626116943 | Centrosomal protein |
| TraesCS5A01G446400.1 | chr5A:626118590..626121272 | Nodulin-like / Major Facilitator Superfamily protein |
| TraesCS5A01G446500.1 | chr5A:626228378..626233779 | Cytochrome P450 |
| TraesCS5A01G446600.1 | chr5A:626258054..626261113 | Gag polyprotein |
| TraesCS5A01G446700.1 | chr5A:626272971..626275671 | krueppel-like factor |
| TraesCS5A01G446800.1 | chr5A:626275616..626279223 | WD repeat-containing protein 1 |
| TraesCS5A01G446900.1 | chr5A:626285929..626288078 | Disease resistance protein (NBS-LRR class) family |
| TraesCS5A01G447000.1 | chr5A:626607075..626610705 | Zinc finger CCCH domain-containing protein 4 |
| TraesCS5A01G447100.1 | chr5A:626688136..626701543 | Trihelix transcription factor ASIL2 |

| | | |
|---|---|---|
| TraesCS5A01G447200.1 | chr5A:626966020..626975963 | Pleiotropic drug resistance ABC transporter |
| TraesCS5A01G447300.1 | chr5A:626989267..626992571 | Succinate dehydrogenase assembly factor 2, mitochondrial |
| TraesCS5A01G447300.2 | chr5A:626989267..626992571 | Succinate dehydrogenase assembly factor 2, mitochondrial |
| TraesCS5A01G447400.1 | chr5A:626992791..626997213 | Aminopeptidase |
| TraesCS5A01G447500.1 | chr5A:627514101..627514412 | DNA gyrase subunit A |
| TraesCS5A01G447600.1 | chr5A:627665486..627671277 | Zinc finger CCCH domain-containing protein 4 |
| TraesCS5A01G447700.1 | chr5A:627784747..627789827 | F-box SKIP23-like protein (DUF295) |
| TraesCS5A01G447800.1 | chr5A:629247282..629250890 | Zinc finger CCCH domain-containing protein 4 |
| TraesCS5A01G447900.1 | chr5A:629372270..629375715 | Zinc finger CCCH domain-containing protein 4 |
| TraesCS5A01G447900.2 | chr5A:629372268..629375715 | Zinc finger CCCH domain-containing protein 4 |
| TraesCS5A01G448000.1 | chr5A:629697510..629699077 | B12D protein |

**Appendix 7.2** Results of the putative candidate genes associated with BYD resistance on chromosome 6A.

| Annotated gene ID | Physical position | Putative function |
|---|---|---|
| TraesCS6A01G009500.1 | chr6A:4151556..4182072 | Disease resistance protein (TIR-NBS-LRR class) family |
| TraesCS6A01G009600.1 | chr6A:4176648..4178238 | disease resistance protein (TIR-NBS-LRR class) |
| TraesCS6A01G009700.1 | chr6A:4181990..4209986 | Disease resistance protein (TIR-NBS-LRR class) family |
| TraesCS6A01G009800.1 | chr6A:4541303..4541940 | Cytochrome c biogenesis ATP-binding export protein CcmA |
| TraesCS6A01G009900.1 | chr6A:4570477..4576753 | Disease resistance protein (TIR-NBS-LRR class) family |
| TraesCS6A01G009900.2 | chr6A:4570477..4576753 | Disease resistance protein (TIR-NBS-LRR class) family |
| TraesCS6A01G010000.1 | chr6A:4578277..4580581 | F-box family protein |
| TraesCS6A01G010100.1 | chr6A:4592563..4597619 | Disease resistance protein (TIR-NBS-LRR class) family |
| TraesCS6A01G010200.1 | chr6A:4678487..4684680 | Protein kinase, putative |
| TraesCS6A01G010300.1 | chr6A:4708160..4712078 | Disease resistance protein RPM1 |
| TraesCS6A01G010400.1 | chr6A:4767512..4773630 | Disease resistance protein (TIR-NBS-LRR class) family |
| TraesCS6A01G010500.1 | chr6A:5035108..5036518 | F-box domain containing protein |
| TraesCS6A01G010600.1 | chr6A:5048122..5049710 | Mitochondrial metalloendopeptidase OMA1 |
| TraesCS6A01G010700.1 | chr6A:5062481..5074465 | Myb/SANT-like DNA-binding domain protein |
| TraesCS6A01G010800.1 | chr6A:5062481..5074465 | F-box domain containing protein |
| TraesCS6A01G010900.1 | chr6A:5106501..5109993 | S-adenosylmethionine decarboxylase proenzyme |
| TraesCS6A01G011000.1 | chr6A:5134428..5135920 | F-box domain containing protein |
| TraesCS6A01G011100.1 | chr6A:5206480..5207583 | INO80 complex subunit D |

| | | |
|---|---|---|
| TraesCS6A01G011200.1 | chr6A:5221215..5222782 | F-box domain containing protein |
| TraesCS6A01G011300.1 | chr6A:5294067..5297246 | RING/U-box superfamily protein |
| TraesCS6A01G011400.1 | chr6A:5318936..5322513 | RNA binding protein |
| TraesCS6A01G011500.1 | chr6A:5323416..5324863 | stress response NST1-like protein |
| TraesCS6A01G011600.1 | chr6A:5326049..5329033 | MADS-box transcription factor 21 |
| TraesCS6A01G011700.1 | chr6A:5358050..5364845 | MADS-box transcription factor |
| TraesCS6A01G011700.2 | chr6A:5358050..5364845 | MADS box transcription factor |
| TraesCS6A01G011800.1 | chr6A:5465228..5480984 | Two-component response regulator |
| TraesCS6A01G011900.1 | chr6A:5491660..5505112 | Two-component response regulator |
| TraesCS6A01G012000.1 | chr6A:5541558..5558395 | Two-component response regulator |
| TraesCS6A01G012100.1 | chr6A:5602824..5606348 | Cytochrome P450 |
| TraesCS6A01G012200.1 | chr6A:5602862..5607327 | RNA binding protein |
| TraesCS6A01G012300.1 | chr6A:5671518..5676470 | Cytochrome P450 |
| TraesCS6A01G012400.1 | chr6A:5774197..5776510 | Cytochrome P450 |
| TraesCS6A01G012500.1 | chr6A:5856594..5857535 | 4,5-dioxygenase-like protein |
| TraesCS6A01G012600.1 | chr6A:5861481..5863576 | Flavonoid 3'-hydroxylase |
| TraesCS6A01G012600.2 | chr6A:5861481..5863576 | Flavonoid 3'-hydroxylase |
| TraesCS6A01G012700.1 | chr6A:5872609..5874397 | Isoflavone reductase-like protein |
| TraesCS6A01G012700.2 | chr6A:5872609..5874397 | Isoflavone reductase-like protein |
| TraesCS6A01G012800.1 | chr6A:5900684..5904508 | Leucine-rich repeat receptor-like protein kinase family protein |
| TraesCS6A01G012900.1 | chr6A:5916455..5919579 | Leucine-rich repeat receptor-like protein kinase family protein |

| | | |
|---|---|---|
| TraesCS6A01G013000.1 | chr6A:5916455..5919579 | Leucine-rich repeat receptor-like protein kinase family protein |
| TraesCS6A01G013100.1 | chr6A:5939421..5942887 | Leucine-rich repeat receptor-like protein kinase family protein |
| TraesCS6A01G013200.1 | chr6A:6189852..6193345 | Trihelix transcription factor GT-2 |
| TraesCS6A01G013200.2 | chr6A:6189852..6193345 | Trihelix transcription factor GT-2 |
| TraesCS6A01G013300.1 | chr6A:6562688..6569872 | Two-component response regulator |
| TraesCS6A01G013400.1 | chr6A:6596643..6609089 | Centromere O |
| TraesCS6A01G013500.1 | chr6A:6698824..6701555 | Leucine-rich repeat receptor-like protein kinase family protein |
| TraesCS6A01G013600.1 | chr6A:6712515..6725497 | Nitric oxide synthase 1 |
| TraesCS6A01G013600.2 | chr6A:6712515..6725497 | Nitric oxide synthase 1 |
| TraesCS6A01G013700.1 | chr6A:6727556..6735295 | Ubiquitin family protein |
| TraesCS6A01G013700.2 | chr6A:6727545..6735295 | Ubiquitin family protein |
| TraesCS6A01G013800.1 | chr6A:6739669..6740877 | Large proline-rich protein BAG6 |
| TraesCS6A01G013900.1 | chr6A:6741669..6742488 | Outer envelope protein 61 |
| TraesCS6A01G014000.1 | chr6A:6804410..6809656 | RING/FYVE/PHD zinc finger superfamily protein |
| TraesCS6A01G014100.1 | chr6A:6890782..6893547 | Polygalacturonase non-catalytic protein |
| TraesCS6A01G014200.1 | chr6A:6894798..6896403 | BTB/POZ domain containing protein, expressed |
| TraesCS6A01G014300.1 | chr6A:6972081..6977742 | Disease resistance protein (NBS-LRR class) family |
| TraesCS6A01G014400.1 | chr6A:7030741..7031500 | Eukaryotic translation initiation factor 4E-1B |
| TraesCS6A01G014500.1 | chr6A:7079402..7081939 | Pentatricopeptide repeat-containing protein |

| | | |
|---|---|---|
| TraesCS6A01G014600.1 | chr6A:7079402..7081939 | Pentatricopeptide repeat-containing protein |
| TraesCS6A01G014700.1 | chr6A:7145822..7150913 | Disease resistance protein (NBS-LRR class) family |
| TraesCS6A01G014800.1 | chr6A:7173458..7176807 | Pentatricopeptide repeat-containing protein |
| TraesCS6A01G014900.1 | chr6A:7184445..7187060 | Pentatricopeptide repeat-containing protein |
| TraesCS6A01G015000.1 | chr6A:7184445..7187060 | Pentatricopeptide repeat-containing protein |
| TraesCS6A01G015100.1 | chr6A:7200746..7203849 | Acetate kinase |
| TraesCS6A01G015200.1 | chr6A:7394093..7395820 | Mitochondrial transcription termination factor-like |
| TraesCS6A01G015300.1 | chr6A:7397016..7398587 | Chalcone synthase |
| TraesCS6A01G015400.1 | chr6A:7595345..7597090 | O-methyltransferase family protein |
| TraesCS6A01G015500.1 | chr6A:7697399..7699079 | Chalcone synthase |
| TraesCS6A01G015600.1 | chr6A:7723151..7724785 | Peptidylprolyl isomerase |
| TraesCS6A01G015700.1 | chr6A:7731502..7733340 | F-box protein |
| TraesCS6A01G015800.1 | chr6A:7969403..7977263 | Cytosine-specific methyltransferase |
| TraesCS6A01G015800.2 | chr6A:7969403..7977263 | Cytosine-specific methyltransferase |
| TraesCS6A01G015900.1 | chr6A:7977624..7982869 | Mitochondrial transcription termination factor-like |
| TraesCS6A01G015900.2 | chr6A:7977624..7982869 | Mitochondrial transcription termination factor-like |
| TraesCS6A01G016000.1 | chr6A:7986639..7988442 | Mitochondrial transcription termination factor-like |
| TraesCS6A01G016000.2 | chr6A:7986664..7990096 | Mitochondrial transcription termination factor-like |
| TraesCS6A01G016100.1 | chr6A:7991124..7992848 | Mitochondrial transcription termination factor-like |

**Appendix 7.3** Results of the putative candidate genes associated with BYD resistance on chromosome 7A.

| Annotated gene ID | Physical position | Putative function |
|---|---|---|
| TraesCS7A01G550000.1 | chr7A:724118037..724121551 | Receptor-kinase, putative |
| TraesCS7A01G550100.1 | chr7A:724122281..724126185 | Receptor-kinase, putative |
| TraesCS7A01G550200.1 | chr7A:724126436..724134579 | Hexosyltransferase |
| TraesCS7A01G550300.1 | chr7A:724134645..724138669 | Protein kinase |
| TraesCS7A01G550400.1 | chr7A:724240498..724251628 | transducin family protein / WD-40 repeat family protein |
| TraesCS7A01G550500.1 | chr7A:724257706..724262982 | ATP-dependent Clp protease proteolytic subunit |
| TraesCS7A01G550600.1 | chr7A:724296067..724299935 | Cysteine protease, putative |
| TraesCS7A01G550700.1 | chr7A:724368618..724375220 | ATP-dependent Clp protease proteolytic subunit |
| TraesCS7A01G550800.1 | chr7A:724457573..724458747 | BTB/POZ domain containing protein |
| TraesCS7A01G550900.1 | chr7A:724652812..724655026 | Glycosyltransferase family 92 protein |
| TraesCS7A01G551000.1 | chr7A:724735305..724736518 | AGAMOUS-like MADS-box protein |
| TraesCS7A01G551100.1 | chr7A:724946126..724949518 | Lysine ketoglutarate reductase trans-splicing protein (DUF707) |
| TraesCS7A01G551200.1 | chr7A:724963594..724966117 | Ser/Thr-rich protein T10 in DGCR region |
| TraesCS7A01G551300.1 | chr7A:724966126..724968146 | tRNA-splicing endonuclease, putative |
| TraesCS7A01G551400.1 | chr7A:725033817..725034863 | Ring finger protein, putative |
| TraesCS7A01G551500.1 | chr7A:725134765..725138178 | Lysine ketoglutarate reductase trans-splicing protein (DUF707) |
| TraesCS7A01G551600.1 | chr7A:725150694..725153990 | Lysine ketoglutarate reductase trans-splicing protein (DUF707) |
| TraesCS7A01G551700.1 | chr7A:725161672..725165676 | Core-2/I-branching beta-1,6-N- |

| | | |
|---|---|---|
| | | acetylglucosaminyltransferase family protein |
| TraesCS7A01G551800.1 | chr7A:725210864..725212958 | Xylosyltransferase 2 |
| TraesCS7A01G551900.1 | chr7A:725224333..725228684 | Disease resistance protein (NBS-LRR class) family |
| TraesCS7A01G552000.1 | chr7A:725229997..725234418 | Flavin-containing monooxygenase |
| TraesCS7A01G552000.2 | chr7A:725229997..725234418 | Flavin-containing monooxygenase |
| TraesCS7A01G552100.1 | chr7A:725303048..725304086 | Chitinase |
| TraesCS7A01G552200.1 | chr7A:725310782..725311652 | Flavin-containing monooxygenase |
| TraesCS7A01G552300.1 | chr7A:725324742..725325555 | Ankyrin repeat family protein |
| TraesCS7A01G552400.1 | chr7A:725433279..725435275 | High mobility group protein |
| TraesCS7A01G552400.2 | chr7A:725433279..725435275 | High mobility group protein |
| TraesCS7A01G552500.1 | chr7A:725439886..725442464 | Phosphatidylinositol N-acetyglucosaminlytransferase subunit P-like protein |
| TraesCS7A01G552600.1 | chr7A:725455166..725462149 | Copalyl diphosphate synthase-like protein |
| TraesCS7A01G552700.1 | chr7A:725551016..725557120 | Copalyl diphosphate synthase-like protein |
| TraesCS7A01G552800.1 | chr7A:725612386..725614212 | BURP domain protein RD23 |
| TraesCS7A01G552900.1 | chr7A:725720398..725721913 | F-box protein |
| TraesCS7A01G553000.1 | chr7A:725911992..725915961 | Aquaporin |
| TraesCS7A01G553100.1 | chr7A:725927237..725936221 | Calcium-binding EF hand protein-like |
| TraesCS7A01G553200.1 | chr7A:725999864..726002549 | Kinesin-like |
| TraesCS7A01G553300.1 | chr7A:726207461..726208617 | Retrotransposon protein, putative, unclassified |
| TraesCS7A01G553400.1 | chr7A:726392726..726407507 | F-box family protein |
| TraesCS7A01G553500.1 | chr7A:726422641..726423731 | Heat shock family protein |
| TraesCS7A01G553600.1 | chr7A:726467009..726470618 | Disease resistance protein RPM2 |
| TraesCS7A01G553700.1 | chr7A:726474481..726476933 | Disease resistance protein (NBS-LRR class) family |

| | | |
|---|---|---|
| TraesCS7A01G553800.1 | chr7A:726481932..726485460 | MYB-related protein |
| TraesCS7A01G553800.2 | chr7A:726481932..726485460 | MYB-related protein |
| TraesCS7A01G553800.3 | chr7A:726481932..726485460 | MYB-related protein |
| TraesCS7A01G553800.4 | chr7A:726481932..726485460 | MYB-related protein |
| TraesCS7A01G553800.5 | chr7A:726481932..726485460 | MYB-related protein |
| TraesCS7A01G553900.1 | chr7A:726604240..726606051 | Nodulin-like / Major Facilitator Superfamily protein |
| TraesCS7A01G554000.1 | chr7A:726658197..726659637 | SPX domain-containing protein |
| TraesCS7A01G554100.1 | chr7A:726670199..726675914 | SPX domain-containing protein |
| TraesCS7A01G554200.1 | chr7A:726683194..726686323 | NBS-LRR disease resistance protein-like protein |
| TraesCS7A01G554300.1 | chr7A:726803088..726805732 | transmembrane protein, putative (DUF594) |
| TraesCS7A01G554400.1 | chr7A:726808169..726812267 | Disease resistance protein (TIR-NBS-LRR class) |
| TraesCS7A01G554500.1 | chr7A:726856879..726859615 | Protein DETOXIFICATION |
| TraesCS7A01G554600.1 | chr7A:726905255..726908253 | eukaryotic release factor 1-2 |
| TraesCS7A01G554600.2 | chr7A:726905255..726908253 | inner centromere protein, ARK-binding region protein |
| TraesCS7A01G554700.1 | chr7A:726979477..726983477 | Dirigent protein |
| TraesCS7A01G554800.1 | chr7A:727001580..727003036 | SPX domain-containing protein |
| TraesCS7A01G554900.1 | chr7A:727005660..727006338 | Bifunctional lycopene cyclase/phytoene synthase |
| TraesCS7A01G555000.1 | chr7A:727052378..727054257 | Dirigent protein |
| TraesCS7A01G555100.1 | chr7A:727123303..727125182 | Dirigent protein |
| TraesCS7A01G555200.1 | chr7A:727220813..727221872 | Disease resistance protein (NBS-LRR class) family |
| TraesCS7A01G555300.1 | chr7A:727228606..727232504 | NBS-LRR disease resistance protein-like protein |
| TraesCS7A01G555400.1 | chr7A:727228606..727232545 | disease resistance protein (TIR-NBS-LRR class) |

| | | |
|---|---|---|
| TraesCS7A01G555500.1 | chr7A:727276539..727278612 | Nodulin-like / Major Facilitator Superfamily protein |
| TraesCS7A01G555600.1 | chr7A:727429803..727434384 | Cellulose synthase-like protein |
| TraesCS7A01G555700.1 | chr7A:727443573..727458353 | Callose synthase |
| TraesCS7A01G555800.1 | chr7A:727532448..727533452 | Ring finger protein |
| TraesCS7A01G555900.1 | chr7A:727580553..727582075 | Ring finger protein |
| TraesCS7A01G556000.1 | chr7A:727583653..727584475 | Ring finger protein |
| TraesCS7A01G556100.1 | chr7A:727710402..727711217 | Ring finger protein |
| TraesCS7A01G556200.1 | chr7A:727721797..727722420 | Ring finger protein |
| TraesCS7A01G556300.1 | chr7A:727773606..727774142 | LONGIFOLIA protein |
| TraesCS7A01G556400.1 | chr7A:727773543..727775141 | Ring finger protein |
| TraesCS7A01G556500.1 | chr7A:727787920..727788837 | Ring finger protein |
| TraesCS7A01G556600.1 | chr7A:727800729..727801497 | Ring finger protein |
| TraesCS7A01G556700.1 | chr7A:727832854..727833773 | Ring finger protein |
| TraesCS7A01G556800.1 | chr7A:727847914..727850945 | Monosaccharide-sensing protein 3 |
| TraesCS7A01G556900.1 | chr7A:727858566..727860463 | Hemoglobin and hemoglobin-haptoglobin-binding protein A |
| TraesCS7A01G557000.1 | chr7A:727897452..727899776 | thiamine-phosphate synthase |
| TraesCS7A01G557100.1 | chr7A:729261444..729265741 | Chloroplastic group IIA intron splicing facilitator CRS1, chloroplastic |
| TraesCS7A01G557200.1 | chr7A:729274169..729277337 | F-box family protein |
| TraesCS7A01G557300.1 | chr7A:729397165..729401510 | Phytoene synthase |
| TraesCS7A01G557300.2 | chr7A:729397165..729401925 | Phytoene synthase |
| TraesCS7A01G557400.1 | chr7A:729935428..729942300 | Protein argonaute |
| TraesCS7A01G557500.1 | chr7A:730377686..730378608 | Dynamin-related protein |
| TraesCS7A01G557600.1 | chr7A:730425419..730429850 | Sucrose synthase |
| TraesCS7A01G557700.1 | chr7A:730430413..730435061 | Disease resistance protein (NBS-LRR class) family |
| TraesCS7A01G557700.2 | chr7A:730430495..730434493 | NBS-LRR disease resistance protein |

| | | |
|---|---|---|
| TraesCS7A01G557800.1 | chr7A:730454306..730464650 | Chromodomain-helicase-DNA-binding family protein |
| TraesCS7A01G557800.2 | chr7A:730454306..730464650 | Chromodomain-helicase-DNA-binding family protein |
| TraesCS7A01G557900.1 | chr7A:730504549..730505579 | 'putative beta-1,3-glucanase |
| TraesCS7A01G558000.1 | chr7A:730700569..730701877 | MADS-box transcription factor family protein |
| TraesCS7A01G558100.1 | chr7A:730709676..730713717 | PPR containing protein |
| TraesCS7A01G558200.1 | chr7A:730713938..730715773 | 4-hydroxy-4-methyl-2-oxoglutarate aldolase |
| TraesCS7A01G558300.1 | chr7A:730717955..730719746 | 4-hydroxy-4-methyl-2-oxoglutarate aldolase |
| TraesCS7A01G558400.1 | chr7A:730721963..730723237 | UDP-glycosyltransferase |
| TraesCS7A01G558500.1 | chr7A:730749195..730749978 | Thaumatin-like protein |
| TraesCS7A01G558600.1 | chr7A:730764173..730766208 | Glycosyltransferase |
| TraesCS7A01G558700.1 | chr7A:730853498..730860159 | basic helix-loop-helix (bHLH) DNA-binding superfamily protein |
| TraesCS7A01G558800.1 | chr7A:730889242..730903126 | Disease resistance protein-like |
| TraesCS7A01G558900.1 | chr7A:731000691..731003363 | F-box family protein |
| TraesCS7A01G559000.1 | chr7A:731011764..731012396 | F-box domain containing protein |
| TraesCS7A01G559100.1 | chr7A:731096027..731097492 | F-box family protein |
| TraesCS7A01G559200.1 | chr7A:731211846..731221195 | F-box domain containing protein |
| TraesCS7A01G559300.1 | chr7A:731263610..731270840 | DUF789 family protein |
| TraesCS7A01G559300.2 | chr7A:731263787..731270840 | Postreplication repair E3 ubiquitin-protein ligase rad19 |
| TraesCS7A01G559300.3 | chr7A:731263787..731270840 | Cellulase (glycosyl hydrolase family 5) protein |
| TraesCS7A01G559400.1 | chr7A:731270752..731275310 | Delta(24)-sterol reductase |
| TraesCS7A01G559500.1 | chr7A:731388301..731389139 | AGAMOUS-like MADS-box protein |
| TraesCS7A01G559600.1 | chr7A:731568425..731571469 | F-box family protein |

| | | |
|---|---|---|
| TraesCS7A01G559700.1 | chr7A:731613032..731616091 | F-box domain containing protein |
| TraesCS7A01G559800.1 | chr7A:731697964..731704077 | Nuclear factor Y B subunit |
| TraesCS7A01G559900.1 | chr7A:731741462..731743685 | F-box family protein |
| TraesCS7A01G560000.1 | chr7A:731882423..731883149 | Dehydrin |
| TraesCS7A01G560100.1 | chr7A:731962146..731966220 | Glucuronoxylan 4-O-methyltransferase |
| TraesCS7A01G560100.2 | chr7A:731962213..731966220 | Glucuronoxylan 4-O-methyltransferase |
| TraesCS7A01G560200.1 | chr7A:732052696..732055490 | Photosystem II stability/assembly factor HCF137 |
| TraesCS7A01G560300.1 | chr7A:732080920..732082975 | tRNA dimethylallyltransferase |
| TraesCS7A01G560400.1 | chr7A:732087180..732090198 | Peptidoglycan-binding LysM domain protein, putative |
| TraesCS7A01G560500.1 | chr7A:732100935..732102560 | Prokaryotic ubiquitin-like protein Pup |
| TraesCS7A01G560600.1 | chr7A:732155959..732158583 | Leucine-rich repeat receptor-like protein kinase family protein, putative |
| TraesCS7A01G560700.1 | chr7A:732352495..732353543 | Late embryogenesis abundant protein |
| TraesCS7A01G560800.1 | chr7A:732360188..732360792 | selection/upkeep of intraepithelial T-cells protein |
| TraesCS7A01G560900.1 | chr7A:732378707..732379297 | GRF zinc finger family protein |
| TraesCS7A01G561000.1 | chr7A:732401834..732402806 | AGAMOUS-like MADS-box protein |
| TraesCS7A01G561100.1 | chr7A:732467399..732471064 | Disease resistance protein (NBS-LRR class) family |
| TraesCS7A01G561200.1 | chr7A:732504661..732508514 | Disease resistance protein (NBS-LRR class) family |
| TraesCS7A01G561200.2 | chr7A:732504661..732508514 | Disease resistance protein (NBS-LRR class) family |
| TraesCS7A01G561300.1 | chr7A:732506981..732515286 | receptor kinase 2 |

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|---|---|---|
| TraesCS7A01G561400.1 | chr7A:732586985..732588719 | Cysteine protease, putative |
| TraesCS7A01G561500.1 | chr7A:732596871..732602616 | DNA-binding protein RHL2 |
| TraesCS7A01G561600.1 | chr7A:732797946..732802031 | Transmembrane protein 54 |
| TraesCS7A01G561700.1 | chr7A:732802032..732805405 | NBS-LRR disease resistance protein |
| TraesCS7A01G561800.1 | chr7A:732913767..732918931 | Thyroxine 5-deiodinase |
| TraesCS7A01G561800.2 | chr7A:732913767..732918914 | Elongation factor P--(R)-beta-lysine ligase |
| TraesCS7A01G561900.1 | chr7A:732919905..732923140 | NBS-LRR disease resistance protein |
| TraesCS7A01G561900.2 | chr7A:732919437..732923990 | NBS-LRR disease resistance protein |
| TraesCS7A01G561900.3 | chr7A:732919437..732923990 | NBS-LRR disease resistance protein |
| TraesCS7A01G561900.4 | chr7A:732919905..732923990 | NBS-LRR disease resistance protein |
| TraesCS7A01G561900.5 | chr7A:732919437..732923990 | NBS-LRR disease resistance protein |
| TraesCS7A01G561900.6 | chr7A:732919437..732923990 | NBS-LRR disease resistance protein |
| TraesCS7A01G562000.1 | chr7A:732933020..732944412 | NBS-LRR disease resistance protein |
| TraesCS7A01G562100.1 | chr7A:732933020..732944457 | Thioredoxin |
| TraesCS7A01G562200.1 | chr7A:732971732..732972993 | F-box protein family-like protein |
| TraesCS7A01G562300.1 | chr7A:733084963..733086451 | Gamma-tubulin complex component |
| TraesCS7A01G562400.1 | chr7A:733212764..733220645 | Disease resistance protein RPM2 |
| TraesCS7A01G562500.1 | chr7A:733273911..733278209 | NBS-LRR disease resistance protein |
| TraesCS7A01G562600.1 | chr7A:733328894..733336912 | Prolyl oligopeptidase family protein |
| TraesCS7A01G562700.1 | chr7A:733337713..733340168 | Coiled-coil domain-containing protein 13 |

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|---|---|---|
| TraesCS7A01G562700.2 | chr7A:733337713..733340217 | Coiled-coil domain-containing protein 13 |
| TraesCS7A01G562800.1 | chr7A:733340220..733351536 | Disease resistance protein RPM2 |
| TraesCS7A01G562800.2 | chr7A:733340220..733351536 | Disease resistance protein RPM2 |
| TraesCS7A01G562900.1 | chr7A:733362302..733368413 | Disease resistance protein RPM2 |
| TraesCS7A01G562900.2 | chr7A:733362302..733368413 | Disease resistance protein RPM2 |
| TraesCS7A01G563000.1 | chr7A:733373577..733375195 | Sucrose synthase 4 |
| TraesCS7A01G563100.1 | chr7A:733432995..733439786 | Receptor-like protein kinase |
| TraesCS7A01G563100.2 | chr7A:733432995..733439786 | Receptor-like protein kinase |
| TraesCS7A01G563200.1 | chr7A:733498845..733500506 | Lecithin-cholesterol acyltransferase-like 2 |
| TraesCS7A01G563300.1 | chr7A:733513123..733514194 | Pathogenesis-related (PR)-10-related norcoclaurine synthase-like protein |
| TraesCS7A01G563400.1 | chr7A:733529378..733529884 | Chlorophyll a-b binding protein, chloroplastic |
| TraesCS7A01G563500.1 | chr7A:733530122..733530980 | transmembrane protein, putative (DUF679) |
| TraesCS7A01G563600.1 | chr7A:733569791..733572048 | Thioredoxin |
| TraesCS7A01G563700.1 | chr7A:733575844..733576355 | RING/U-box superfamily protein |
| TraesCS7A01G563800.1 | chr7A:733610017..733614394 | F-box family protein |
| TraesCS7A01G563900.1 | chr7A:733664942..733668517 | F-box domain containing protein |
| TraesCS7A01G564000.1 | chr7A:733775137..733778785 | Disease resistance protein (NBS-LRR class) family |
| TraesCS7A01G564100.1 | chr7A:733780944..733783532 | F-box domain containing protein |